PROCEEDINGS OF THE 1992 SUGAR PROCESSING RESEARCH CONFERENCE

SEPTEMBER 27-29, 1992 NEW ORLEANS, LOUISIANA

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Sponsored by
Sugar Processing Research Institute, Inc.

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PREFACE

The 1992 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference was sponsored by Sugar Processing Research Institute, Inc. (SPRI). The Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, contributed in kind to the organization of the Conference.

The program for this conference was arranged by Margaret A. Clarke. The Conference Coordinator was Shirley T. Saucier. These Proceedings were edited by Margaret A. Clarke with editorial assistant Beryl Ann Borel.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the sixth issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Before 1986, Proceedings were published by the Agricultural Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by the Sugar Processing Research Institute.

Margaret A. Clarke
Managing Director
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CHEMICAL AND ENZYMIC TRANSFORMATIONS OF SUCROSE

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ABSTRACT

Structurally and functionally, sucrose is a unique sugar amongst
all carbohydrates. Its chemistry has only been recently explored
and has led to the development of a number of products of commer-
cial importance. This lecture will deal mainly with the work that
was carried out at the Philip Lyle Memorial Research Centre, Tate
& Lyle plc, Reading.

Recognizing the problem in performing selective chemical reactions
with sucrose, we adopted specific protection and deprotection
strategies for the eight chemically different hydroxyl groups, of
the sucrose molecule. The three primary hydroxyl group (C-6, 6' and
1') were blocked using such selective reagents as triphenyl
chloromethane (trityl groups), tertiary butyl dimethyl silyl
chloride and tert-butyldiphenylsilyl chloride. The secondary
hydroxyl groups (C-2,3,4,3',4') were subsequently acetylated or
benzoylated. The silyl and trityl groups were then selectively
removed to regenerate the hydroxyl groups for further chemical
manipulations.

Another approach for selective protection was to exploit the unique
conformational arrangements of the hydroxyl groups in the sucrose.
For example, in acetalation, reaction using 2,2-dimethoxypropane in
DMF under acidic conditions gave the 4,6-monocyclic and the
4,6:1',2-dicyclic acetals. These acyclic acetal derivatives then
led to many specific compounds of sucrose.

Enzymes are known to catalyze selective reaction in carbohydrates.
We have explored the use of lipases in organic solvent systems to
derivatise sucrose in a selective manner. Acetylation of sucrose
with isopropenyl acetate in DMF in the presence of a protease
enzyme gave 4',6-di-0-acetyl sucrose (50% yield) which was an ideal
substrate for the synthesis of "sucralose", a high-intensity
sweetener developed by Tate & Lyle.

Sucrose without any doubt is the "king sugar," and it has enormous
commercial potential not only as a food but also as a feed stock
for chemicals and biologically active materials.

INTRODUCTION

The results of the chemical transformations of sucrose until 1965 were limited to eight research publications describing about 15 well characterised sucrose derivatives (3,4,5,14,20,21,25,26) (Table 1). Progress since then has been rapid, and today there are more than three hundred well identified sucrose derivatives described in the literature (12). In this review, some of the studies which have made significant contribution towards the understanding of the chemistry of sucrose, and have led to products of actual or potential commercial interests, will be described.

STRUCTURE AND CONFORMATION

Sucrose (I, β-D-fructofuranosyl α-D-glucopyranoside), is a non-reducing disaccharide. It contains eight hydroxyl groups, of which three are primary (6,6' and 1') and the remaining five are secondary. The primary hydroxyl groups have been found to react preferentially, in particular the hydroxyl groups at 6 and 6' positions.

The neutron diffraction studies (6) on solid crystalline sucrose revealed two strong intramolecular hydrogen bonds (O-2.... HO-1'and O-5.... HO-6') which serve to hold the molecule in a well-ordered, rigid conformation in which the two rings are approximately at right angles (I), and in which the pyranosyl residue adopts a $^4C_1$1 conformation and the furanoside ring a $^3T_4$ twist. Apart from HO-4, all the hydroxyl groups are intermolecularly hydrogen bonded.

According to hard sphere calculations and ^1H- and ^{13}C-nmr studies, Bock and Lemieux (1) have shown that in dilute aqueous solution sucrose had a similar conformation to the crystal structure but with only one intramolecular H-bond. Using X-ray and Raman spectroscopy, Mathlouthi et al. (22) found no evidence of intramolecular H-bonds in dilute solution (<0.7M) but observed that as concentration was increased to a saturated solution, intramolecular H-bonds were formed, resembling those in the crystal structure. The conclusion that the conformation of aqueous sucrose was dependent on concentration was not supported by the ^{13}C-nmr relaxation studies (24). Application of secondary isotope multiplet partially labelled entities to nmr in deuterated dimethylsulphoxide revealed the presence of two different conformations with intramolecular H-bonds from either the 1'-OH or the 3'-OH of the fructofuranoside as donors to the acceptor 2-OH of the glucopyranosyl residue (8) (III). These conformations are in competitive equilibrium, with 1'OH.....2-O, predominating over the 3'-OH.....2-O, in the ratio of 2:1, respectively. The inter-unit hydrogen bond is strengthened by co-operative hydrogen bonding from

the neighbouring hydroxyl groups in both glucosyl and fructoside residues.

Trityl Ethers

Sucrose on treatment with 4 molar equivalents of chlorotriphenyl-methane (trityl) chloride in pyridine afforded, after acetylation and chromatography, 6,1',6'-tri- (IV) and 6,6'-di- (V) O-tritylsu-croses in 50 and 30%, respectively (15). Compound IV is an intermediate in the synthesise of sucralose (11), a high-intensity sweetener developed by Tate & Lyle plc and Johnson & Johnson of the USA. Detritylation of IV with aqueous acetic acid followed by acetyl migration from C-4 to C-6 gave (V), which on chlorination with thionyl chloride, pyridine and trichloroethane gave, after deacetylation, sucralose (4,1',6'-trichloro-4,1',6'-trideoxy-galactosucrose).

Cyclic Acetals

The most significant development in the chemistry of sucrose has been the synthesis of cyclic acetals which had defied preparation, despite many attempts, until 1974. The first synthesis of 4,6-0-benzylidenesucrose (16) (VI, 35% yield) was achieved from the reaction of sucrose with α,α-dibromotoluene in pyridine. Since then many new novel acetalating reagents have been used to give a variety of sucrose acetals. Treatment of sucrose with 2,2-dimethoxypropane, N,N-dimethylformamide and toluene-p-sulphonic acid gave 4,6-0- isopropylidene (VIII) and 4,6:1',2-di-0- iso-propylidene (IX) derivatives in good yields (17). The unique 8-membered 2,1'-cyclic acetal bridges the two rings in sucrose, is more stable to acid than the 4,6-acetal linkage and has been effective in providing access to selective reactions at 2 and 1' positions in sucrose.

The diphenylsilylene derivatives were synthesised by the same approach as above, using 2,2-dimethoxydiphenylsilane, N,N-dimethyl-formamide and toluene-p-sulphonic acid to give the 2,1'- (X) and 2,1':6,6'-di- (XI) -O-(diphenylsilylene) derivatives in 45% and 10% yield, respectively (13). These compounds gave access to new positions in the sucrose molecule for chemical and enzymic transformations.

The First S_N2 Displacement Reaction at C-2 Position in Sucrose and in Methyl α-D-glucopyranoside

The formation of a transition state at C-2 to allow an S_N2 displacement reaction in methyl α-D-glucopyranoside was considered

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to be not possible because of the unfavourable dipole- dipole interactions due to the ring oxygen and the C-1 oxygen. However, when methyl 3-0-benzoyl-4,6-0-benzylidene-α-D-glucopyranoside 2-chlorosulphate (XII) was treated with lithium chloride in hexamethylphosphoric triamide, it gave with inversion of configuration at C-2 the corresponding 2-chloro-manno derivative (18) (XIII). Similar treatment of the 2,1'bis- (chlorosulphate) (XIV) with lithium chloride led to the 2,1'-manno derivative (XV). Since then there have appeared many reports in the carbohydrate literature to support that an S_N2 displacement reaction in an alkyl α-D-glucopyranoside is possible.

Cyclic Orthoesters

4,6-Cyclic orthoester derivatives of sucrose are valuable intermediates for the synthesis of 6-ester derivatives of sucrose. Garegg et al. have described the use of this approach in their elegant synthesis of 6-O-acetyl-2,3,4-tri-O-(3S-methylpentanoyl)-sucrose, a precusor of tobacco flavour (10).

Sucrose 6-acetate was required as an intermediate for the synthesis of sucralose. Treatment of sucrose with trimethylorthoacetate, N,N,-dimethylformamide and toluene-p-sulphonic acid followed by acid hydrolysis gave the 6-0-acetylsucrose (XVI) as the major and the 4-acetate (XVII) as the minor component (30). Compound XVII under went acetyl migration from C-4 to C-6, when treated with an organic base such as t-butylamine in N,N-dimethylformamide to give XVI. When the kinetic reagent 2,2-dimethoxyethene was used, instead of trimethylorthoacetate, 4,6-O-(1-methoxyethylidene) sucrose, the intermediate for compound XVI, was obtained in near quantitative yield (19). Selective chlorination of compound XVI gave sucralose (XVIII) in high yield. This approach should offer a more economical route to the synthesis of sucralose.

Enzymic Acylation Reaction

Enzyme catalysed acylation reactions offer considerable promise in the synthesis of specific ester derivatives of sucrose. For example, reaction of sucrose with an activated alkyl ester in N,N-dimethylformamide in the presence of subtilisin gave the 1'-acylate (XIX), which on further treatment with an activated fatty acid ester in acetone in the presence of lipase C. viscosum afforded the 1',6-diester (28) (XX).

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Treatment of sucrose with isopropenylacetate in pyridine in the presence of lipase P Amano gave, after chromatography, 6-O-acetyl-sucrose (XXI, 33%) and 4',6-di-O-acetylsucrose (XXII, 8%). Compound XXII has been obtained in 47% yield by prolong treatment, and has been converted to sucralose (XVIII) in high yield (25).

Enzymic Deacylation Reaction

Partially esterified derivatives of sucrose, not easily accessible by chemical methods, have been prepared using enzymes. Enzymatic hydrolysis of sucrose octaacetate (XXIII) in a phosphate or citrate buffer has led to a series of compounds such as sucrose hepta-acetate 4'-hydroxy (XXIV), sucrose heptaacetate 4-hydroxy (XXV), and sucrose tetraacetate 4,1',6',4'-tetrahydroxy (2) (XXVI). Compounds XXIV and XXVI have been transformed to sucrose tetra-acetate 4'-butyrate 4,1',6'-trihydroxy XXVII, which on chloria-nation followed by deacylation gave sucralose (9) (XVIII).

Some Commercially Important Products from Sugar

The recent chemical and biotechnological advances and the resulting commercial opportunities have influenced the business strategy of the sugar industry. Most of the big sugar companies have now extended their interests beyond sugar into starch, high fructose corn syrup, synthetic and natural high-intensity sweeteners, and other related food products.

Production of industrial ethanol, and chemicals such as acetic acid and acetic anhydride therefrom, from sugar in countries like Brazil, India and Pakistan is continuing. Microbial polys-accharides such as xanthan gum, gellan, and alginates have already demonstrated their commercial potential (31).

Sucralose (11), a high-intensity sweetener from sucrose, is being developed by Tate & Lyle plc and Johnson & Johnson of the USA. It has been approved for use in foods in Canada and is awaiting approval as a food and drink additive by the FDA in the U.S. and health authorities in other countries. Sucralose gives a taste like sucrose, is heat stable, and is 650 time the sweetness of sucrose. The market potential of sucralose can be assessed on the basis of the market size for aspartame which in 1985 was roughly $700 million.

Sucrose mono fatty acid esters have applications in detergents, food and feed, cosmetics, and pharmaceuticals. These esters are produced in Japan on a commercial scale (27). The reaction of sucrose with a triglyceride or a methyl ester of a fatty acid has normally been carried out in an aprotic solvent in the presence of a basic catalyst. In a solvent-less process, sucrose is heated

with stirring, with tallow in the presence of potassium carbonate at 140°C to afford a mixture containing roughly 27% of sucrose monoesters, 3% of the higher esters and mono-, di-, and triglycerides, and 30% soaps. This crude mixture exhibits   notable surface active properties. The increase in the contents of higher esters will impart the mixture emulsifying characteristics. The market for low cost biodegradable surfactants in the treatment of oil spills is considerable.   Major industrial applications for non-toxic and biocompatable sucrose based surfactants can be visualised in the detergent industry, in the area of cosmetics, and in food and feed formulations.

Sucrose polyesters developed by Procter and Gamble under the brand name "Olestra" are neither absorbed or hydrolysed by pancreatic lipases and are therefore classed as low-calorie fats (23).   In addition, these products appear to reduce the level of cholesterol in humans.   The polyesters contain 6-8 fatty acid ester molecules per sucrose, and are prepared by a solventless transesterification process.   Treatment of sucrose with ethyl ester of fatty acids is carried out in the presence of sodium methoxide at temperature range of 100° to 180°C for 14 hours.   The unreacted fatty acid esters and lower substituted sucrose esters are removed by enzymic hydrolysis with lipase. Sucrose polyesters containing five and more fatty acid esters are resistant to lipases.   Since fat consumption in the USA alone is nearly 6 million tonnes a year, only a few percent of "Olestra" would raise the sugar demand by thousands of tonnes.   However, they have yet to be approved by the FDA for human consumption.

Isomaltulose (Palatinose) and Isomalt (Palatinit) are being developed as reduced calorie and non-cariogenic food ingredients. An efficient process for the production of isomaltulose, 6-0-α-D-glucopyranose D-fructose, was developed by Tate & Lyle (7). Isomaltulose is produced on an industrial scale from sucrose using immobilised α-glucosyl transferase from <u>Protaminobacter</u> <u>rubrum</u>. In Japan, palatinose is used as a non-cariogenic sweetener.   In Germany, Südzucker GmbH also produces isomaltulose using a similar technology. They convert isomaltulose into Isomalt® by a catalytic hydrogenation process (29).   The reduced product is a mixture of 6-0-α-D-glucopyranose D-sorbitol and   6-0-α-D-glucopyranose D-mannitol. The sweetness intensity of the mixture is roughly half that of sucrose.

CONCLUSION

New advances in products and processes can only be achieved if sufficient resources are allocated to research and development. Investment in sucrose chemistry research so far has paid, directly or indirectly, large dividends to those who had wisdom and patience

to invest in that business. Our own achievements to list a few are:
- understanding of the fundamental aspects of the chemistry of sucrose.
- sucrose fatty acid esters as surfactants and detergents.
- sucralose, a high-intensity sweetener.
- Talin, a high-intensity sweet protein.
- transformed sugar, a product that has no molasses byproduct.
- microbial polysaccharides, xanthan gum and alginates.
- production of isomaltulose using an immobilised enzyme system.

Sucrose is the "king" of the carbohydrates. It is the only organic compound which is pure, low-cost, and available world wide on a scale of 110 million tonnes a year. Its potential as a raw material for products of commercial importance has been demonstrated but not yet fully realised. The future of sucrose as a chemical feedstock for polymers, for example rigid polyurethane foams, as surfactants and detergents, and as detergent builders, for example sucronic acid as a replacement for sodium tripolyphosphate, holds considerable promise. In the area of food additives, the high cost involved in the toxicology and long lead-time required for regulatory approvals will discourage future developments.

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Table 1. Some characterised sucrose derivatives, review up to 1965

                6,1',6'-Tri-O-tritylsucrose1
                Sucrose octaacetate2
                Sucrose octabenzoate2
                Sucrose octabenzyl ether3
                Sucrose octamethyl ether4
                6,1',6'-Tri-O-tritylsucrose5
                Sucrose Pentaacetate 6,1',6'-trihydroxy6
                Sucrose Pentaacetate 4,1',6'-trihydroxy6
                4,1',6'-trimethylsucrose6
                2,3,4,3',4'-pentamethylsucrose6
                6,1',6'-Tri-O-tosylsucrose7
                6,6'-Di-O-tosylsucrose8
                3,6:1',2:3',6'-Trianhydrosucrose8

Table 2. Sugar based products of commercial importance

                Ethanol and chemicals therefrom
                Sugar based polyurethanes
                Microbial polysaccharides
                    Dextran
                    Xanthan
                    Alginates
                    Gellan
                    Polyfructans
                High-intensity sweeteners
                    Sucralose - "Splenda"
                Surfactants and detergents
                    Sucrose esters
                Low-calorie fats - Sucrose polyesters
                    "Olestra"
                Low-cal low-intensity sweeteners
                    Isomaltulose
                    Isomalt
                    Neosugars
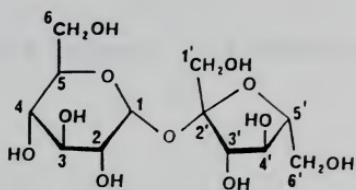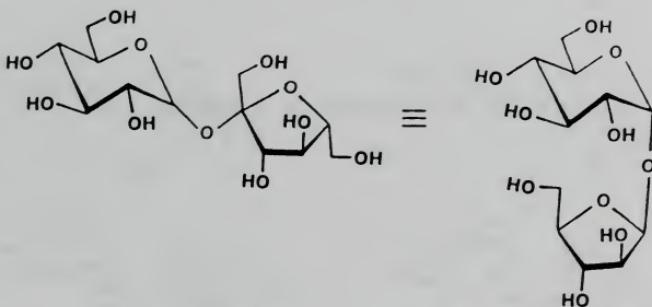
α-D-Glucopyranosyl ´β-D-fructofuranoside
or
β-D-Fructofuranosyl α-D-glucopyranoside

Figure 1
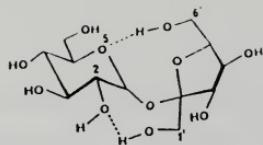
Neutron Diffraction - Crystal Structure

I
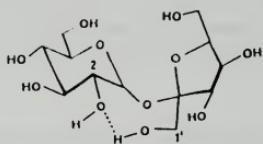
^{1}H, ^{13}C nmr - Structure in Solution

II
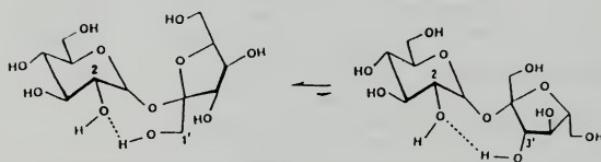
SIMPLE nmr - Structure in Solution

III

Figure 2

12
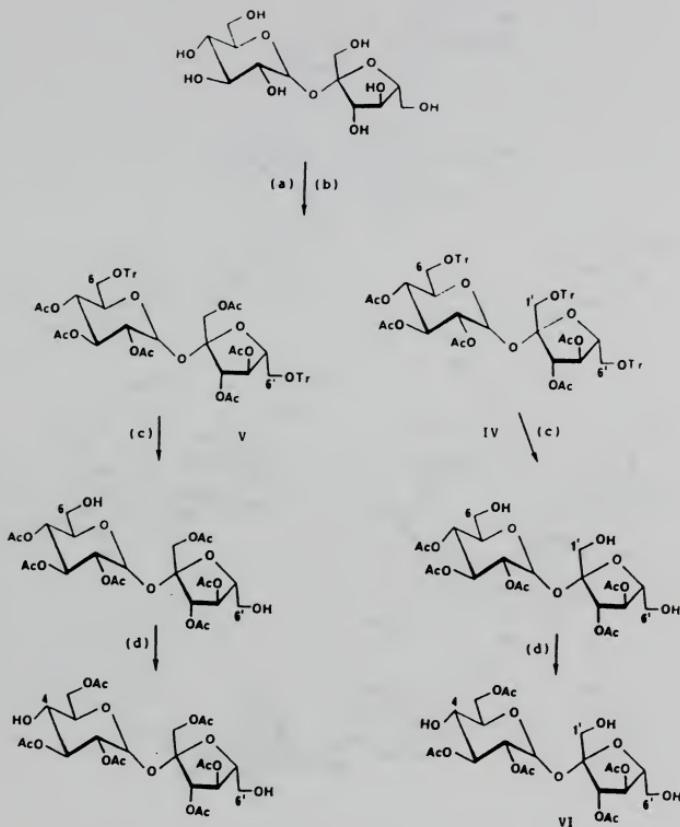
REAGENTS: (C₆H₅)₃CCl, pyridine; (b) Ac₂O, pyridine;
(c) HBr/AcOH, CH₃Cl; (d) Aq.AcOH
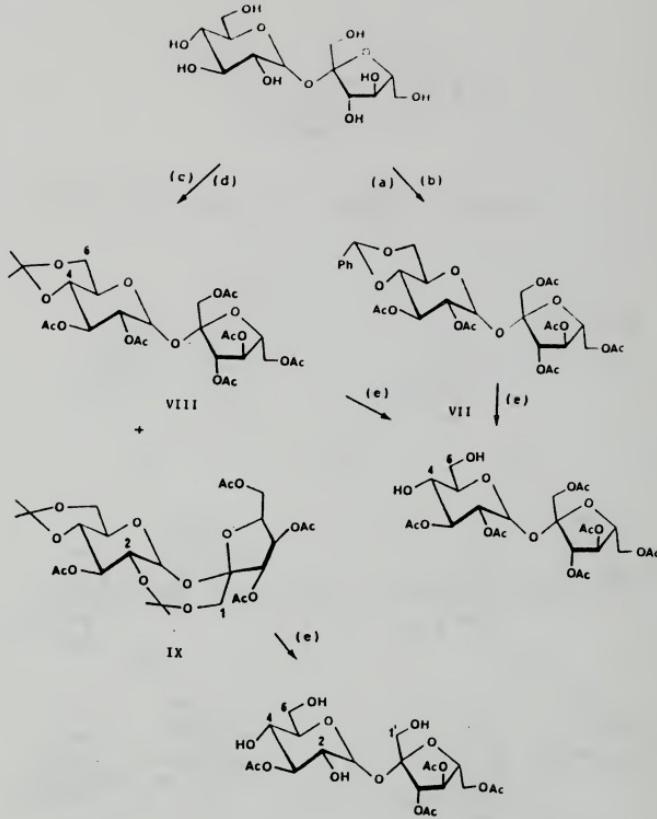
Figure 3

REAGENTS: (a) $C_6H_5CHBr_2$, Pyridine, (35%); (b) $C_6H_5CH(OCH_3)_2$, dmf, p-TsOH, (45%); (c) $CH_3C(OCH_3)_2CH_3$, dmf, p-TsOH; (d) $CH_2=CH(OCH_3)CH_3$, dmf, p-TsOH; (e) Aq.AcOH

Figure 4

14
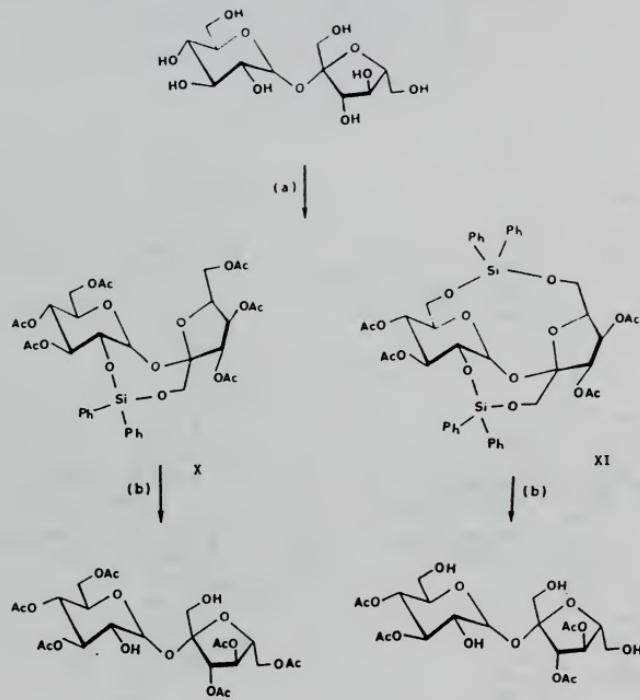
REAGENTS: (a) Ph₂Si(OMe)₂, dmf, p-TsOH; (b) Aq. AcOH

Figure 5
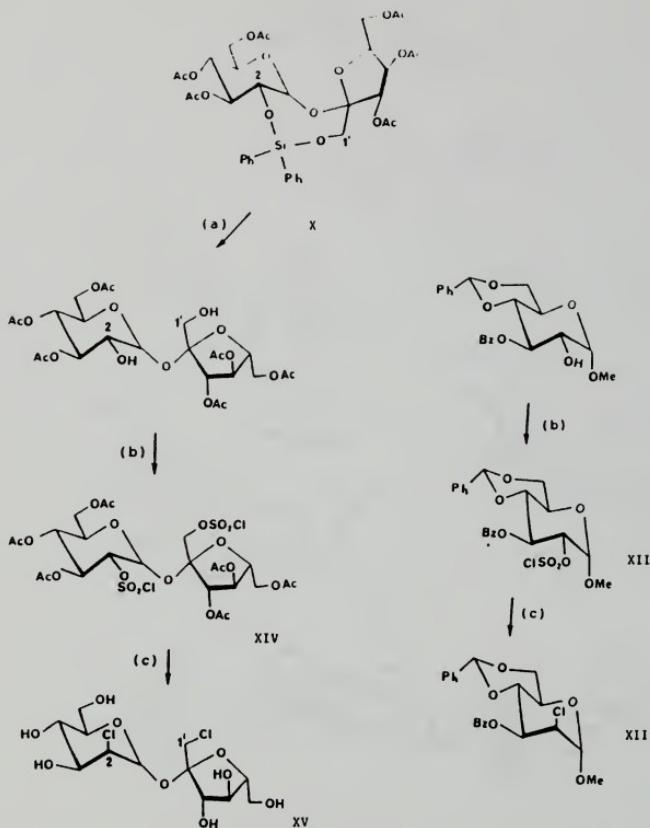
REAGENTS: (a) Aq. AcOH; (b) SO_2Cl_2, Pyridine, $CHCl_3$; (c) LiCl, dmf; (d) NaN_3, dmf
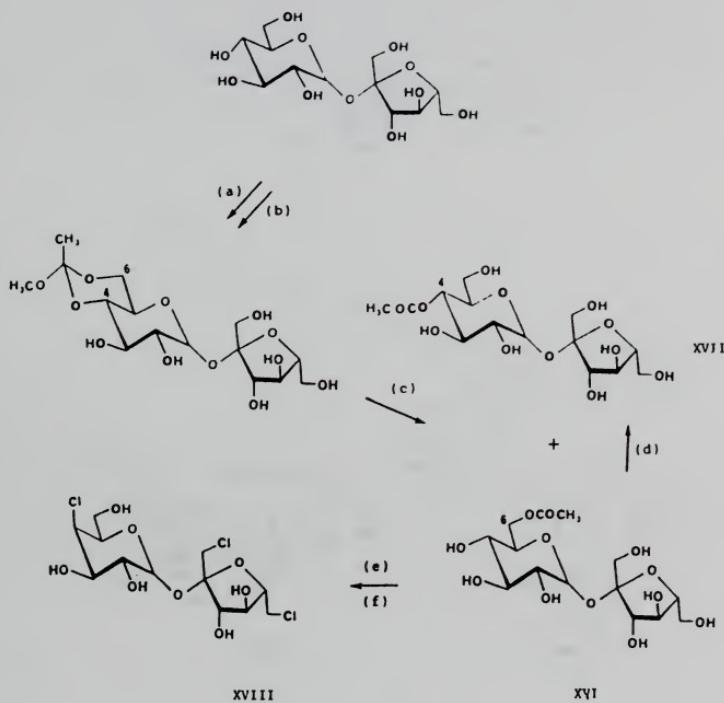
Figure 6

REAGENTS: (a) MeC(OMe)$_3$, dmf, p-TsOH; (b) CH$_2$=C(Me)OMe, dmf, p-TsOH; (c) Aq. AcOH; (d) t-BuNH$_2$; (e) SOCl$_2$, Pyridine, Trichloroethane; (f) NaOMe, MeOH

Figure 7
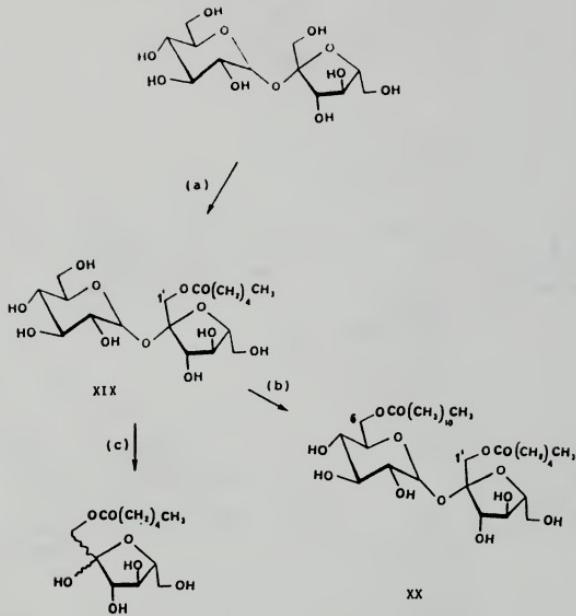
REAGENTS: (a) $CH_3(CH_2)_4COOCH_2CF_3$, dmf, Protease N, 45°C, 2 d (45%); (b) Lipase C.viscosum, Acetone, (45%); (c) α-D-Glucosidase, Phosphate buffer, pH 5.0, r.t.

Figure 8
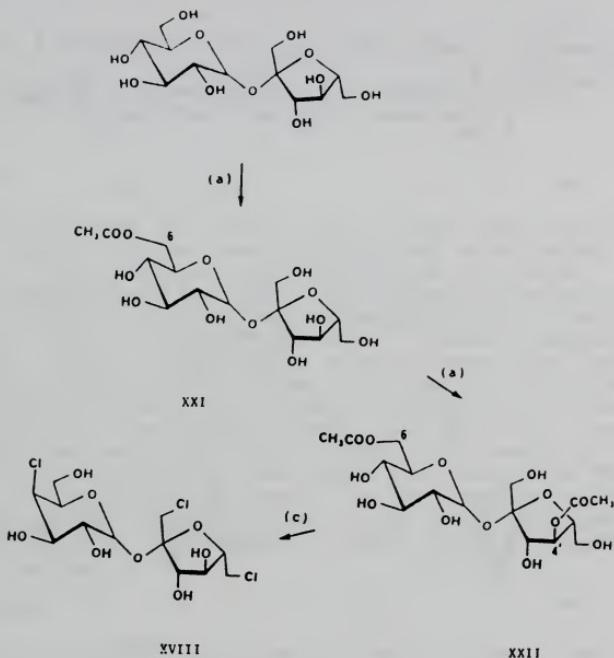
REAGENTS: (a)"Lipase P Amano", LPL05518, $CH_2=C(OAc)CH_3$,
Pyridine, (33% mono, 8% di); (b) Lipase P Amano,
Trichloroethyl butyrate, Pyridine; (c) $SOCl_2$,
Pyridine, trichloroethane

Figure 9
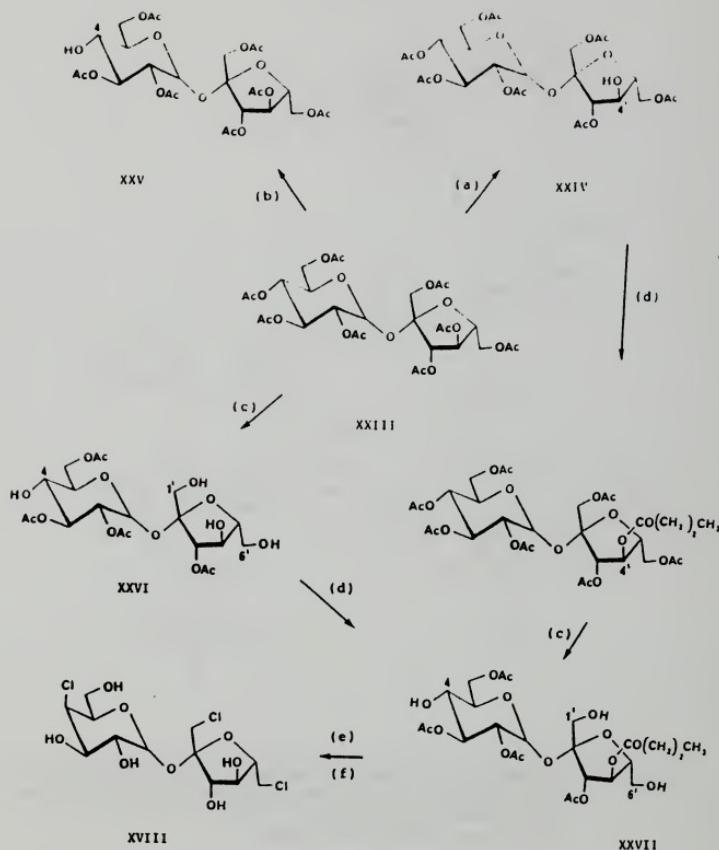
REAGENTS: (a) Pancrelipase, Citrate buffer, pH 5.0, r.t.,
24 h, (60%); (b) Stachyase, Phosphate buffer, 30°C
72 h, (20%); (c) Yeast esterase, Citrate buffer,
(30%); (d) Lipase P Amano, Pyridine,
Trichloroethyl butyrate, 60°C, (60%); (e) SOCl$_2$,
Pyridine, trichloroethane; (f) NaOMe, MeOH

Figure 10

STANDARDS FOR GRANULAR CANE SUGAR FOR USE IN CARBONATED BEVERAGES

Daniel W. Bena, Gregory Radko, and Joseph B. Kuntz,
Pepsi-Cola International, Valhalla, New York

ABSTRACT

Sixty-five samples of granular cane sugar from various worldwide
sources were evaluated analytically and were tested for sensory
impact in both lemon-lime and cola-based beverages.   Statistical
Pearson correlations were calculated to relate the analytical and
sensory data. The highest correlation was between the turbidity of
the sugar and an "unrefined sugar" sensory characteristic in
beverage.   Other significant correlations that exist between
analytical parameters and sensory attributes are listed in Table 4
in the "Results" section of this manuscript. As a result of these
correlations, a new specification for granular cane sugar was
developed that is statistically-based, with ranges for each major
analytical parameter that are founded in correlated sensory data.
Because of the potential impact to the quality of the finished
beverage, it is important that sugar purchased internationally
conform to the new standards set forth in this document.

INTRODUCTION

Historically, little published data exist in the beverage industry
relating analytical evaluation of cane sugar to its impact on
specific sensory attributes in beverage. The impetus behind this
project was first, the limited availability of high quality
granular cane sugar internationally, and second, the need for a
sugar specification founded on sensory data (i.e., "safe" from the
standpoint of minimal impact on the finished beverage), and also
allowing greater flexibility for sourcing from international
markets.   In general, the disparity in quality between domestic
(U.S.A.) granular cane sugar and that available internationally has
long been of concern to the beverage industry.   "Bottler White"
standards for granulated sugar as set forth by the National Soft
Drink Association (4) require such stringent standards for color
and ash that many international supplies do not conform.   Conse-
quently, costly sugar treatment by the bottling/canning facility
becomes mandatory. In addition, the N.S.D.A. does not address any
quantitative standard for turbidity, another subject of importance
to soft-drink manufacturers--primarily with regard to the visual
aesthetics of the finished beverage.

In this study, sixty-five samples were sourced from China, India, Mexico, Argentina, Venezuela, and the Middle East (these samples are not representative of the entire sugar supply in each country). Each sugar sample was prepared into both a lemon-lime and cola-based beverage matrix in order to utilize the different sensitivities of the two flavor systems. Each beverage was aged and subjected to rigorous sensory evaluation by an expert panel. The focus was the presence/absence of each of 12 sensory characteristics (qualitative component) in addition to the relative degree (none, low, medium, high) of each characteristic (semi-quantitative component). The specific sensory attributes evaluated are listed in Table 1.

MATERIALS AND METHODS

I. Sensory Testing:

The flavor profiling utilized an expert panel of 5 to 10 trained individuals to determine the qualitative and semi-quantitative sensory impact that a sugar can impart to the beverage. The training of the panel included tasting several chemical sensory standards (for example, cis-3-hexenol for "green" characteristics) that had been added to control beverages. This established a common vocabulary of descriptors to use during the "qualitative" component of flavor profiling. In addition, admixed ratios of a poor quality raw sugar to a control sugar were also prepared to help standardize the panel with respect to the "quantitative" component of flavor profiling. That is, once the particular sensory characteristic is perceived, to what relative degree is it present (low, medium, high)? All tastings included a control beverage prepared with American "Bottler Grade" granulated sugar. All samples were prepared into both lemon-lime and cola-based beverages and aged at ambient temperature for a minimum of three weeks. Each test beverage was then tasted with regard to the presence/absence and degree of the twelve sensory characteristics listed in Table 1.

II. Analytical Testing

The analytical tests that were performed are summarized in Table 2.

Although not all sugar samples were subjected to every analysis, each sugar received a minimum of color, turbidity, ash, anion and cation profiling, floc potential, and sensory testing.

A. Color and Turbidity

Color was performed using a modification of the recommended I.C.U.M.S.A. method (5), which employs filtration through a 0.45μ

filter disc followed by spectrophotometric absorbance at 420 nm. There was no pH adjustment, and the concentration of the sample sugar solution was 23.7 Brix.   No filter aid was employed. Absorbance was read on a Shimadzu UV-160 Double Beam Spectrophotometer.   Turbidity was similarly performed, with a difference in absorbance readings at 720 nm recorded before and after the filtration.

B.   Anion/Cation Profiling

Anion/Cation Profiling was performed on a Waters Ion Chromatography System consisting of a Waters 431 Conductivity Detector, Waters 490 E Programmable Multiwavelength Detector, Waters Wisp 712, Waters 510 High Pressure Pump, and IC-Pak anion and cation analysis columns (with guard columns). A borate/gluconate buffer system was used for anion detection, and a nitric acid/calcium disodium edetate buffer for cation quantitation. Specific protocols can be found in the Waters Ion Chromatography Cookbook (6).

C.   Elemental (metals) Analysis

Elemental analysis was performed by Atomic Absorption Spectroscopy with graphite furnace utilizing a Perkin-Elmer   5000 Automatic Burner Control and Perkin-Elmer HGA-500 Programmer.

D.   Conductivity Ash

Conductivity Ash was performed on a Yellow Springs Instrument Model 35 Conductivity Meter, at 25°C, with a conductivity probe cell constant of 1 cm^{-1}.

E.   Other Analyses

Floc Potential was characterized by attempting to simulate adverse syrup conditions of heat and acidity in order to catalyze the formation of the floc.   Other parameters were evaluated using standard in-house methods of analysis.

RESULTS

A tabular summary of results for 14 key analytical parameters is shown in Table 3. The results are grouped according to geographic area of submission; the means, ranges, and number of samples in each group are included.

The correlations expressed (r) represent Pearson correlations for comparison of parametric and non-parametric data. When employing Pearson correlation data, a value of 0.40 or above is generally considered a significant correlation. For comparison purposes, a correlation of 0.68 is considered very high (3).

DISCUSSION OF RESULTS

The nutritive sweeteners used as a major component of many soft drinks can, depending on their quality, have appreciable impact on the sensory profile of the finished beverage. Granular cane sugar is no exception. As a result of this project, it was found that cane sugar has the potential of eliciting several different sensory characteristics from a final beverage. The most frequently perceived attributes were "unrefined sugar, burnt, and molasses" in the cola-based matrix, and "musty/dirty, fruity, and metallic" in the lemon-lime based matrix.

The data collected for each sensory characteristic were then systematically compared to each of the analytical parameters evaluated. As shown, the highest correlation was between the analytical parameter of turbidity and the sensory characteristic of "unrefined sugar" flavor in the lemon-lime beverage matrix. A similar correlation resulted between the same two measures in the cola-based beverages. Several other analytical parameters were significantly correlated with sensory attributes, and are summarized in Table 4.

Given this data, an explanation as to the components of turbidity in granular cane sugar was sought. The literature contains limited information regarding turbidity, but in summary, lipid, protein, starch, silica (1), suspended minerals, cane waxes, polysaccharides, and higher molecular weight polyphenols are implicated. In addition to turbidity, the high molecular weight polyphenols have also been associated with effecting "unrefined" sensory notes in the sugars in which they are contained (2). This was confirmed in beverage by "adding back" the non-dialyzable material from milled sugar samples into prepared control drinks. After aging, the bitter, unrefined sensory characteristics were duplicated in the beverages under test.

The remainder of the sensory and the analytical data was compared for each sample from the standpoint of total sensory scores. That is, the overall sensory results for each sample were determined to be either acceptable or unacceptable, and the corresponding analytical ranges for each chemical parameter were then developed into the final specification for granular cane sugar. It is this set of standards (see Table 5) against which it is required that granular cane sugar for soft drinks must be purchased internationally.

CONCLUSION

Supported by the Pearson correlations listed in the text, this project reaffirms the idea that the quality of the granular cane sugar can have appreciable sensory impact on the finished beverage in which it is used. An understanding of the data that exists regarding cane sugar turbidity components also lends confidence to the findings of this project that turbidity is highly correlated with "unrefined sugar" characteristics in some soft-drinks.

In addition to the increased importance of turbidity, the equally important roles of color and ash as indicators of granular cane sugar quality were also confirmed. It is not the intent of this paper to tout turbidity alone as the all-encompassing measure of cane sugar quality. Rather, the authors hope to convey the collective importance of turbidity, color, and ash as a "triad" of quality measures for granular cane sugar.

Finally, as a result of this project, standards have been set forth for the purchase of granular cane sugar internationally. It is required that the sugar purchase by our bottling/canning facilities conform to the standards listed in Table 5. In addition to the sensory and analytic parameters referenced above, the material must also be acceptable microbiologically.

Note: Mention of trade names is for descriptive purposes only, and does not imply endorsement by PepsiCo. We thank Judy Ho-Silver for the statistical analysis, and Sharon Rine for the sensory training.

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Table 1. Specific Sensory Attributes Evaluated in Test Beverages

Unrefined Sugar	Burnt
Licorice	Molasses
Sweet	Tart
Salty	Musty/Dirty
Green	Fruity
Metallic	Other

Table 2. Analytical Parameters Evaluated for Granular Cane Sugar Samples

Ammonium	Arsenic	Ash, Conductivity
Bromide	Calcium	Chloride
Color	Copper	Iron
Fluoride	Indicator Value	Lithium
LC-RI	LC-UV	Magnesium
Manganese	Nitrate	Nitrite
Lead	Phosphate	Polarimetry
Potassium	Sodium	Sulfate
Sulfide	Sulfite	Thiosulfate
Turbidity	Floc Potential	

LC-RI = High Performance Liquid Chromatography with Refractive Index Detection

LC-UV = High performance Liquid Chromatography with Ultraviolet Detection

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Table 3. Summary of Analytic Results by Geographic Region
(Numbers in parentheses () designate the number of samples involved from each region, and are the same for each parameter.)

Parameter	Site	Average	Minimum	Maximum
Ash, Conduc.	Argentina(9)	0.033	0 006	0.113
	China (10)	0.043	0.001	0.074
	India (10)	0.041	0.015	0.063
	Mexico (13)	0.022	0.005	0.055
	Middle East(13)	0.017	0.006	0.037
	Venezuela (10)	0.034	0.022	0.056
Calcium	Argentina	49.48	6.22	142.20
	China	84.66	9.61	146.50
	India	84.55	0.00	172.70
	Mexico	56 45	17.94	120.50
	Middle East	14.49	0.77	64.05
	Venezuela	62.46	0.00	106.83
Chloride	Argentina	5.98	0.00	33.08
	China	40.14	2.70	92.11
	India	23.89	0 00	51.80
	Mexico	4.85	0.00	17.58
	Middle East	11.84	0.01	27.77
	Venezuela	21.84	0.00	100.00
Color	Argentina	81	47	121
	China	105	65	144
	India	121	60	252
	Mexico	86	33	151
	Middle East	60	25	134
	Venezuela	98	55	153
Indicator Val.	Argentine	4.0	1.0	13.0
	China	6.1	2.5	9.0
	India	3.8	1.0	6.2
	Mexico	3.7	1.3	7.5
	Middle East	1.8	1.0	3.0
	Venezuela	2.7	1.8	3.5
Nitrate	Argentina	1.21	0.00	10.17
	China	0.67	0.00	6.00
	India	0.77	0.00	7.70
	Mexico	0.00	0.00	0.00
	Middle East	8.02	0.00	14.00
	Venezuela	0.00	0.00	0.00
Nitrite	Argentina	0.00	0.00	0.00
	China	14.10	0.00	94.90
	India	0.26	0.00	2.61
	Mexico	1.03	0.00	7.22
	Middle East	0 00	0.00	0.00
	Venezuela	0.00	0.00	0.00

Parameter	Site	Average	Minimum	Maximum
Phosphate	Argentina	4.51	0 00	40.60
	Chine	2 62	0 00	23.60
	India	0.00	0.00	0 00
	Mexico	0.14	0.00	1.00
	Middle East	7.82	0.00	38 00
	Venezuela	20.53	0.00	55.38
Polarimetry	Argentina	99.0	98 1	99 6
	China	100 0	98 9	101 2
	India	99 4	96 8	101 0
	Mexico	99.6	99 0	100 3
	Middle East	100 7	99 ?	101 0
	Venezuela	99 5	98 4	100 8
Potassium	Argentina	80.68	8 33	314 00
	China	107 19	11 40	308.00
	India	69 10	0 00	115 50
	Mexico	16.33	0 00	103.40
	Middle East	70.46	3.22	96.00
	Venezuela	27.52	0 00	104 70
Sodium	Argentina	33.08	2 88	221.00
	China	7.82	3 70	28.00
	India	13.86	0.72	25.50
	Mexico	3.92	0.66	17.30
	Middle East	5.64	3 03	8.05
	Venezuela	2.36	0.00	6.53
Sulfate	Argentina	107.73	14.22	528.56
	China	227.65	0.00	503.10
	India	190.30	65 55	407.30
	Mexico	128.06	4.77	294 40
	Middle East	31.98	0.00	125.50
	Venezuela	183.53	113.50	355.60
Sulfite	Argentina	0.80	0.00	7.20
	China	0.00	0.00	0.00
	India	20.10	0.00	88.03
	Mexico	0 00	0.00	0.00
	Middle East	0.00	0.00	0.00
	Venezuela	0.00	0.00	0.00
Turbidity	Argentina	30	9	78
	China	44	27	60
	India	68	26	124
	Mexico	30	7	68
	Middle East	25	12	51
	Venezuela	36	5	110

All of the anion, cation, and metal results are expressed in units of parts per million (ppm). The conductivity ash is expressed as a percentage, and color and turbidity are expressed in "modified I.C.U.M.S.A. units," as described in the Materials and Methods section of this manuscript.

Table 4. Other Significant Correlations Between Analytical Measures and Sensory Attributes

Analytical Measure	Sensory Attribute	Correlation (r)
for cola-based matrix:		
Ash, Conductivity	Unrefined/Burnt/Fruity	0.47/0.40/0.40
Chloride	Metallic	0.41
Magnesium	Unrefined/Molasses/Fruity	0.42/0.46/0.43
Potassium	Unrefined/Burnt/Molasses	0.58/0.55/0.44
Sodium	Unrefined/Burnt/Sweet	0.41/0.68/0,65
Turbidity	Unrefined/Burnt/Molasses	0.58/0.40/0.47
For lemon-lime matrix:		
Ammonium	Green/Fruity	0.51/0.48
Ash, Conductivity	Unrefined/Molasses/Musty/ Fruity	0.45/0.50/0.41/0.46
Calcium	Fruity	0.41
Chloride	Salty	0.42
Color	Unrefined/Musty	0.52/0.56
Potassium	Unrefined/Molasses/Fruity	0.42/0.47/0.65
Sodium	Licorice/Tart/Salty/Musty/ Green/Metallic	0.56/0.40/0.44/0.42/ 0.48/0.42
Turbidity	Unrefined/Burnt/Molasses/ Musty	0.68/0.56/0.55/0.47

(Ho-Silver, Reference 4).

Table 5. Comparison of Pepsi Specification and Other Industrially Important
Standards for Granular Cane Sugar

Parameter	Pepsi Spec.	N.S.D.A.	Codex Alimentarius
Ash, Conduct.	0.035% max.	0.015% max.	0.04% max
Color	60 mod I.U. max.	35 R.B.U. max	60 I.U. max.
Turbidity	45 mod I.U. max	20 R.B.U. max	N.S.
Polarization	99.5 to 100.5 S	N.S.	99.7 S min.
Foreign Matter	20 ppm max.	2 ppm max.	N.S.
Beverage Floc	passes test	N.S.	N.S.
Sensory	passes test	passes test	N.S.

Code:

N.S. = not specified

Max. = maximum value allowed

Min. = minimum value allowed

R.B.U. = Reference Basis Units according to N.S.D.A.

I.U. = I.C.U.M.S.A. units

Mod. I.U. = Modified I.C.U.M.S.A. units (see Materials and Methods for
description)

N.S.D.A. = National Soft Drink Association, Specifications for "Bottlers"
Granulated Sugar

Codex Alimentarius = Specifications from Vol. III, Worldwide Specifications for
White Sugar, Specification A

DISCUSSION

Question: Besides sugar, a major ingredient in beverages is water. Do you also have standards for water?

Bena: When I said that the quality of the sugar affects the beverage more than any other single ingredient, that was, of course, exclusive of water. One thing we are finding in the sugar testing is that the variability in sugar quality is the tip of the iceberg compared to variability in water quality.

To answer your question: no, we don't have strict specifications at present for the quality of water going to our bottling plants. We recommend various treatments. We are currently working with water, as we did with sugar, to develop tests. However, many of our bottling plants are franchise owned, so, the best we can do is to make recommendations. Part of our corporate vision is to buy out more and more of these franchises, to increase the number of company owned bottling organizations. When that happens, we'll be able to have more direct influence on water quality, but until then, we must just make recommendations. A lot of those are traditional water quality treatments. We are looking more and more at reverse osmosis and monofiltration - the new membrane technologies.

Question: Do you have specifications for proteins, dextrans and other polysaccharides?

Bena: No. These are factors we investigated over the course of the project. We did not find them to be much problem, and so those factors are not included in the specifications.

Question: Do you have further comments on the color test that you used in the study?

Bena: The only color test that Pepsi has been familiar with for 25 years was the reference base unit (RBU), used by the domestic bottling organization and the National Soft Drink Association.

One of our early observations in the international area was that very few people recognized the RBU. One of the early changes we made at the beginning of this project was to replace the RBU with the ICUMSA unit - at first, a modified ICUMSA unit, because we changed the concentration at which we read color, and read at natural pH without adjusting to pH 7. However, we've realized that our conditions are within the ICUMSA guidelines and so now refer to our color measurement as ICUMSA units.

THE FACTORS INFLUENCING THE TRANSFER OF COLOUR TO SUGAR CRYSTALS

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ABSTRACT

A one-litre laboratory vacuum pan has been used to study the
transfer of colours from syrup to crystals during crystallisation.
Efficiency of the crystal yield was all important in controlling
the transfer of colour.   The transfer in the laboratory pan was
similar to that found in production pans and increased as the syrup
colour decreased.   The molecular weights of colourants were also
important in influencing colour transfer.   The balance of transfer
values was determined with materials from factory A-pans, and from
the early steps in refining, and these transfers and their
importance in refining are presented and discussed.

INTRODUCTION

The production of white granulated sugar is the aim of cane sugar
refineries, and in order to achieve this, refineries employ a
variety of decolourising processes. There has been a great deal of
study over the years on these decolourisation processes, and we are
able to choose and optimise a process to meet the needs of a
refinery to reach a specified white sugar colour.

We decided to study the transfer of colour into the sugar crystals,
and apply this knowledge to optimising processes.   It has become
apparent that certain sugar colours transfer much more readily into
the crystals than others.   This is evident from beet processing
where white sugar can be boiled from liquors of a much higher
colour than in cane.

The aim of this work is to build up an understanding of the colour
transfer in a cane refinery, identify the colours that transfer to
crystals, and modify our processes to remove these colours.   This
paper covers our progress towards the first two aims.

Colour transfer can be defined as the crystal colour divided by the
colour of the syrup from which it was boiled (or the colour of its
massecuite).   Thus if a syrup of 200 ICU is boiled to give a
crystal of 20 ICU, the colour transfer would be 0.1.   The crystal

is assumed to be perfectly affined, with the colour inside the crystal, and not in a syrup film on the surface. To be precise the colour of the liquor from which the crystals are being formed increases during the boiling. Nevertheless for our work we have found the use of the masse colour to be satisfactory.

The physical conditions of crystallization which influence the incorporation of colour into crystals have been studied by Lionnet(4), who examined the influence of stirring, supersaturation, crystal size, and crystallization rate in cane systems. Mantovani (6), has studied the formation of coloured inclusions.

The nature of the coloured and other impurities in the syrups is also important. These were studied by Lionnet (3), who examined the effect of syrup quality; by workers at British Sugar (8), and at SPRI (2), who used gel permeation chromatography to explore the effect of colour molecular weight on colour transfer.

CRYSTALLIZATION METHOD

Crystallizations were carried out in a 1 litre jacketed reaction vessel (Figure 1). Hot water was circulated through the jacket to control the temperature at 70°C. The vessel was fitted with a vacuum gauge and a thermocouple. A paddle stirrer was used with the shaft sealed with a lubricated ground glass sleeve. Vacuum and vapour take off were through a tube leading to a T-piece at the bottom of a condenser. A measuring cylinder below the T-piece collected and measured the condensed water. Vacuum was controlled (at approx 0.25 bar) by an air leak and a restriction in the vacuum line.

The solubilities of syrups to be examined were measured by equilibrating with excess sugar at 70°C and measuring the Brix. Before crystallization a syrup was held at least 10°C above its saturation temperature for 1 hour to dissolve any nuclei, and then evaporated to the required supersaturation before seeding with diluted Ditmar seed slurry. As the crystals grew, the pressure and heating water flow were adjusted to allow water to be taken off from the boiling syrup at a continuously increasing rate, while maintaining a constant boiling temperature. This rate matched the increasing rate of sucrose deposition caused by the increasing crystal surface area.

Crystals were harvested by spinning in a laboratory basket centrifuge, washed twice by spinning after stirring with 65 Brix sucrose solution, and air dried. The colours of the crystals and massecuite were measured.

Typical conditions were:

Seed	1.55 mg in 0.5 ml isopropanol.
Stirring	250 rpm.
Charge size	666 gms solids
Water evaporated	59 ml.
Boiling time	55 min.
Supersaturation	1.125.

Under these conditions approximately 40% crystal yield was obtained (calculated from the Brixes of the massecuite and the mother syrup). The crystal sizes were approx 0.5mm. A 5% relative standard deviation represented the variability of the colour transfer measurements by this method.

THE EFFECT OF CRYSTALLIZATION CONDITIONS

Colour Level

The colour of the feed syrup (or crystallization massecuite) itself had an important effect on colour transfer. At lower colours the colour transfer was higher. This is represented by the curve in Figure 2 which has been fitted to the colour transfers of a series of syrup samples from refinery 4th boilings. These were crystallized at different colour levels obtained by diluting them with white sugar. Shown on the figure are points representing colour transfers measured on actual crystals boiled from the same syrups in the refinery, which indicate that the laboratory method does give the same colour transfer results as the full scale vacuum pan. (The production crystals were affined by washing twice with sugar syrup). Results from different liquors are represented on the graph, giving some variability.

This effect of colour on colour transfers was noted by Chiu & Sloane (1), in their examination of a large number of factory A-pan samples. They coined the term "colour transfer". The effect is also implied in the work published by Lionnet (5), who derived linear equations relating crystal colour to syrup colour.

For the purposes of comparing colour transfers between different systems, the effect of colour itself can be corrected by using a, quadratic equation fitted to the data; but for more certain comparisons, samples should be crystallized at essentially the same colours.

This colour transfer data may also be fitted to a Langmuir Isotherm, with the implication that some sort of adsorption mechanism is operating in the transfer of colours to crystals, but

without specifying the nature of the "surface" onto which colours are adsorbed.

Crystallization Rate

A series of crystallizations were carried out on a single batch of (carbonatated) syrup by systematically varying the conditions listed above. A lower initial supersaturation or a longer boiling time led to a lower colour transfer. Both of these changes in conditions imply a lower crystallization rate. A similar effect of rate was found by Lionnet (4).

A single sample of refinery melter liquor crystallized over different times showed the same behaviour.

Boiling Time	Colour Transfer
55 min	0.075
87 min	0.052
110 min	0.043

Such an effect implies that diffusion plays an important part in the transfer of colours, as well as adsorption.

Stirring

More efficient stirring, using a turbine instead of a paddle, or using a higher stirring rate, gives a reduction in colour transfer. More efficient stirring could reduce colour transfer by reducing crystal pocket content. Where no mechanical stirring is used in production pans a higher ash content in crystals has been observed (9), and attributed to syrup occluded in pockets. However, no firm evidence has been found in our studies to link colour transfer with pockets so an alternative explanation may be needed. The more efficient mass transfer afforded by better stirring could help colours that were already adsorbed onto the crystal surface to move away once they had been displaced by sucrose molecules.

COLOUR TYPES

Work published by SPRI (2) reports the use of gel permeation chromatography to study the types of colour inside a crystal, and compares them to the colour in the liquor from which they have been boiled. The conclusion was that the colours inside the crystal are of a higher molecular weight than those in the liquor.

In our studies on the effects of colour molecular weight, small pilot facilities have been used to ultra-filter melter liquor from

Thames Refinery (liquor direct from the melter, prior to carbonata-
tion). The membranes used were ceramic membranes with pore sizes
of 0.2 μ, and 40 Å. The liquor was first passed through the 0.2 μ
membrane, which is able to remove approximately 25% of the colour
in melter liquor. Once the bulk of the liquor had passed through,
water was added to flush the remaining sugar to leave suspended
solids, colour, and other high molecular weight material present in
the retentate.

The permeate was then passed through the 40 Å membrane, and once
again the remaining sugar was flushed through with water to give a
second retentate, containing mostly colour. The 40 Å membrane
separated approximately 55% of the colour that remained in the
permeate from the 0.2 μ membrane.

The permeates were then crystallized, with granulated sugar being
added to the retentate colour solutions to take them up to a
saturation Brix before crystallization.

Subsequent experiments were somewhat abbreviated from the above
method, as retentate was not collected from the 40 Å membrane in
the second experiment, and in the third only the original melter
and the 0.2 μ permeate were crystallized to obtain colour trans-
fers. In the second and third experiments the feeds and products
were brought to similar colour levels by adding white sugar. This
minimised the effect of colour level on
colour transfer and made comparisons more certain. These results
are shown in Table 1, and in Figure 3.

In each case the permeate has a lower colour transfer than the
original liquor, and the retentate has a higher colour transfer.
When the permeate from the 0.2 μ membrane was passed though the 40
Å membrane, the colour transfer is further reduced, and the
retentate has a higher transfer. If colour transfers are assumed
to be additive, it is possible to perform a colour transfer balance
and predict the crystal colours of the membrane feeds from the
colour transfers of the membrane products.

These experiments clearly show that the higher molecular weight
colours, separated by the membranes, give much higher colour
transfers. This confirms the SPRI work (5), but using crystalli-
sation experiments rather than examining crystal sugars.

Further clues can be found in the visible spectrum of the colours.
This is a smooth curve of absorbance against wavelength. The ratio
of the absorbance at two wavelengths has been called the "N-value"
(7). It is related in a rank order to the molecular weight of
colours. There are theoretical grounds for this and it has been
confirmed experimentally (10). In the membrane experiments most of
the N-values were in the same rank order as the colour transfers,
with permeates being lower than the feeds and retentates higher.

This trend is shown in Figure 4. where the colour transfers have been corrected for the effect of the massecuite colour. Additionally, when crystal colours are high enough for N-values to be determined reliably, their N-value was usually higher than that of the feed syrup, implying that higher molecular weight materials are going into the crystals.

PROCESS EFFECTS

A series of sugar and syrup samples taken from Thames Refinery were crystallized at similar colour levels in order to compare their colour transfers. The samples covered the refining areas from raw sugar to the melter and the Recovery House, and were all taken on the same day, at intervals corresponding to the approximate residence times of the individual process steps. The colour transfers obtained are shown in terms of a simplified refinery flow sheet (Figure 5) where three values are given in each box.

These are the colour of the stream, the colour of the liquor used for the colour transfer experiment (sometimes diluted with granulated white sugar to give a colour much lower than the stream colour), and the measured colour transfer. The following features may be seen.

Raw sugar had a colour transfer of 0.052, but when split into separate fractions, the affined crystal and the syrup coating, it is found that the colour inside the crystal has a much higher colour transfer than the coating syrup, 0.061 compared to 0.028.

Samples taken from the refinery mainstream (affined sugar and melter) liquor have higher colour transfer values than materials from the Recovery side of the process (affination syrup and first crop syrup).

The values for affination syrup and 1st crop syrup are similar and are consistent with the addition of Jet 4 which has a colour transfer of approx 0.032, similar to that of the 4th boilings mentioned earlier.

The transfer value of the 1st crop sugar is relatively high, but as only approx 10% of 1st crop sugar is added to the affined sugar to give the final melter liquor, only a small increase in colour transfer would be expected. These studies show that affined sugar and melter liquor have the same value (within the errors of the method - approx 5%).

The likely behaviour of transferring colours can be considered.

In the factory A-pan, transferring colours will concentrate in the raw crystal and be slightly depleted in the syrup. This depletion will be small and should not affect its composition significantly as only about 1% of colour overall goes into the crystal. This syrup surrounds the raw crystals in the centrifugal after purging.

When the raw crystal is washed some of it will dissolve and dilute the syrup, most of which will be removed. Thus the film around the raw will be slightly less coloured than the mother syrup and have a colour transfer that is a little higher.

During affination in the refinery (which will not be perfect), some of the syrup film will go to the mainstream as a residual film around the affined sugar crystals, reducing its colour transfer slightly. Some of the crystal will dissolve and thus raise the colour transfer of the affination syrup above that of the syrup originally surrounding the crystal.

When the affination syrup (mixed with Jet 4 which has a similar colour transfer) is crystallized in the Recovery 1st crop pan, it should contain slightly more transferring colours than the original feed syrup in the factory A-pan. Thus a 1st crop sugar would be expected to have only a slightly higher colour transfer than a raw or affined sugar. The different values of colour transfer for affined sugar and 1st crop sugar are within ths range seen at Thames refinery for a variety of raws. Affined sugar is often higher than 0.061, as seen in the three values for different melter liquors in Figure 3.

The study was extended to take in a number of A Pan massecuites from two raw factories. The colours in these samples (crystallized at different colour levels) are similar to the refinery materials. The transfers are shown in Figure 6 and form a reasonable curve when plotted against the massecuite colour. Affination syrups from Thames Refinery also fall on this curve. The points designated by a date are from Lionnet's work (5), when he crystallized syrups at different stages of the season and found that mid-season syrups (August to October) gave the lowest colour transfers. They also fit on the curve.

CONCLUSIONS

Our laboratory vacuum pan enables us to obtain realistic values for the transfer of colour from syrups to crystals, and can be used to investigate the effect that processing is likely to have on crystal colours.

Work with membranes has shown that colours with higher molecular weights are more readily incorporated into growing crystals, possibly because they are able to diffuse away only slowly from a growing crystal surface, and are thus more likely to be trapped than smaller colours.

This however does not rule out the influence of other colour properties that may cause colours to be bound adsorptively to crystal surfaces, also hindering their escape during crystalliza- tion. Work by Zaorska (11), indicates that such affinities can occur.

The measured colour transfers of refinery materials fit well with the details of the expected flow of transferring colours during the refining process. Mainstream samples have higher colour transfers than samples from the recovery side of the process. This is to be expected because the transferring colours will have been built into the raw crystals. This shows up the conflicting priorities in refining. When purifying sugars for ash or invert, crystallizing to give a raw sugar in the factory A-Pan is a reasonable step. However, given the objective of producing white refined sugars, effective reduction in colour is not as large as it appears, as the behaviour of transferring colours means that a larger proportion will find its way into the syrups feeding white sugar pans. As these are at low colours, their colour transfer will be high. If only colours with lower transfers were present at this stage then acceptable white sugars could be boiled from higher coloured syrups, reducing the amount of decolourisation needed.

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Table 1. The effect of membrane treatments on colour transfer

	Filtered Syrup Colour	Crystallization Feed		Colour Transfer
		Colour	N-Value	
First Experiment				
Melter	1528	1528	29.8	0.071
0.2 μ retentate		400	36.7	0.140
0.2 μ permeate	1079	956	25.6	0.041
40 Å retentate		882	18.6	0.057
40 Å permeate	500	500	15.1	0.029
Second Experiment				
Melter	1397	435	29.8	0.063
0.2 μ retentate		439	39.1	0.116
0.2 μ permeate	1014	411	25.0	0.039
40 Å permeate	429	441	14.6	0.026
Third Experiment				
Melter	1329	869	29.0	0.084
0.2 μ permeate	1012	783	28.3	0.037

$$\text{N-value} = \frac{[\text{Absorbance at 520nm}]^2}{[\text{Absorbance at 455nm}]^2}$$

Fig 1. LABORATORY VACUUM PAN

The numbered squares represent different batches
of 4th boiling material (Some diluted)

The points P represent production batches from 4th boilings
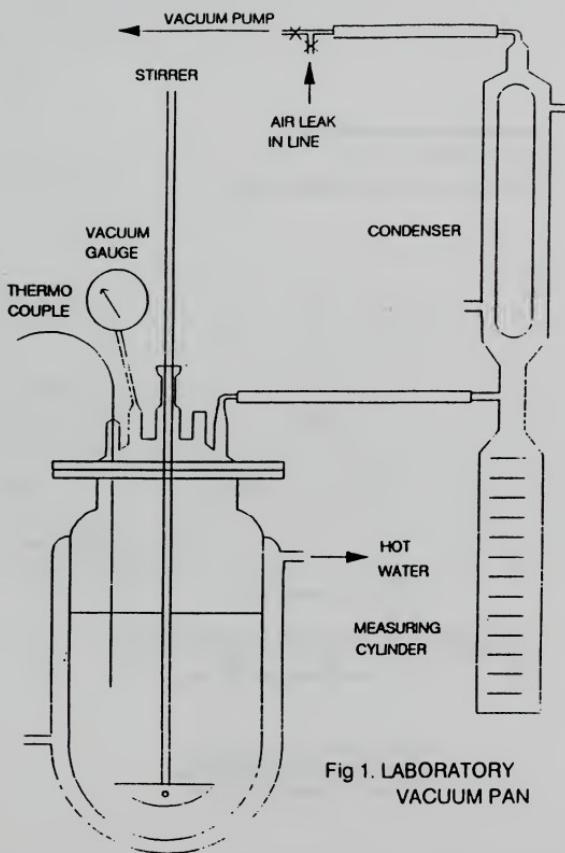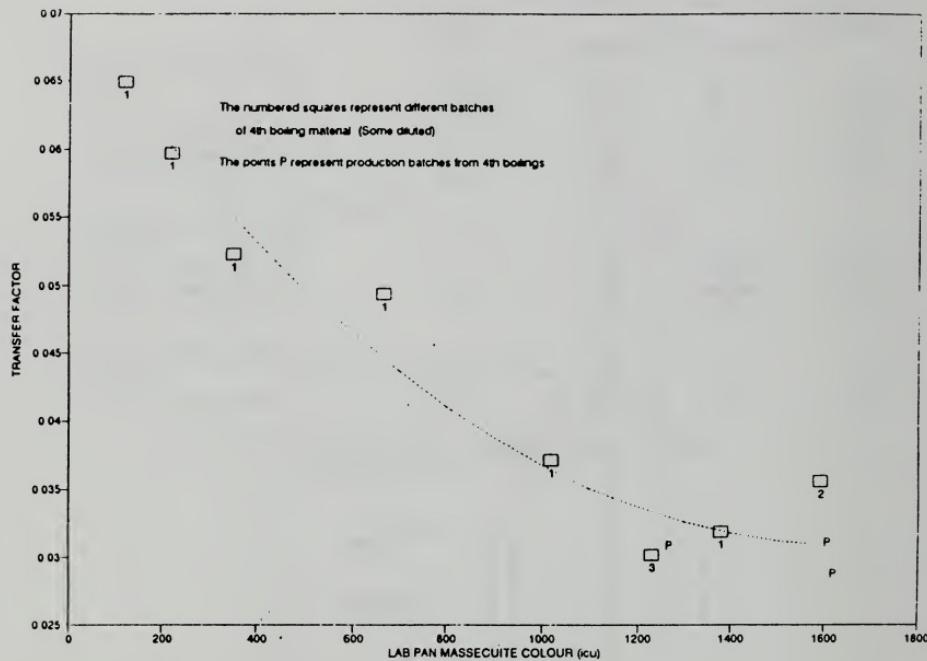
Fig.2 COLOUR TRANSFER
THE EFFECT OF MASSECUITE COLOUR

42
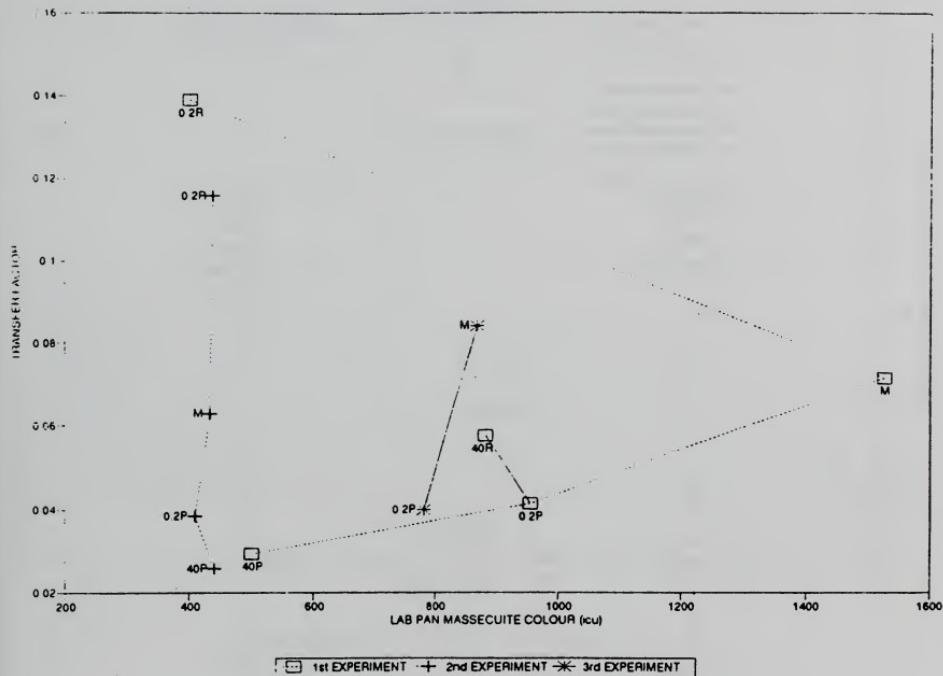
Fig.3 COLOUR TRANSFER
THE EFFECT OF MEMBRANE TREATMENT
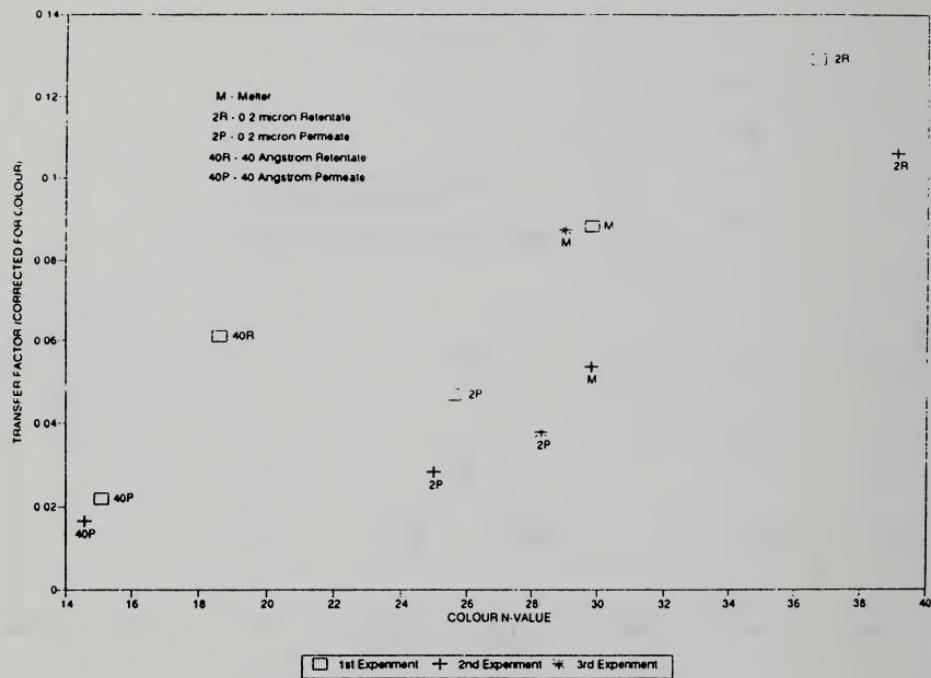
Fig.4 COLOUR TRANSFER
RELATIONSHIP TO COLOUR N-VALUE
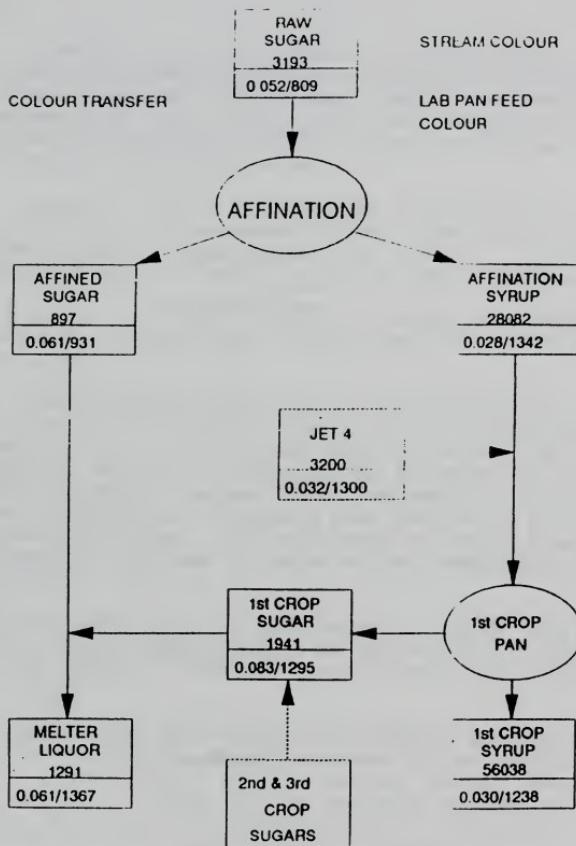
Fig 5. COLOUR TRANSFERS
REFINERY STREAMS
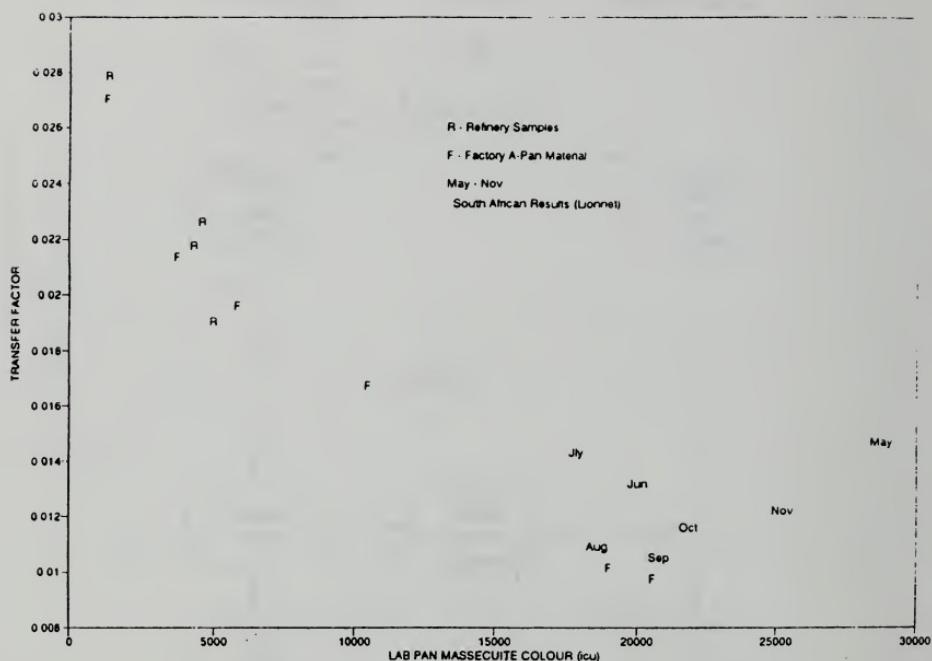
Fig.6 COLOUR TRANSFER
REFINERY AND FACTORY MATERIALS

DISCUSSION

Question: A comment on color transfer during cooling crystallization; from cooling crystallization, we obtained crystals of white sugar practically without inclusions, coming from concentrated raw juice, although raw juice has a very dark colour, almost black. But through cooling crystallization, under particular kinetics, we can obtain practically white sugar.

Question: Thank you for an interesting presentation on an elegant piece of work on color transfer. I'm not clear on one small point: you are talking about color inside the crystal. Is this the case in the factory samples as well as in the laboratory samples?

Donovan: Yes, it is. The factory/refinery samples were washed using the same technique as was used in the laboratory,with 65 Brix white sugar syrup.

Question: A second quick question: we've found that, in agreement with Prof. Mantovani's work, kinetics are vitally important to color transfer. One of the things that we've done in carrying out similar types of work is to examine the transfer of other materials, marker compounds, potassium for example, into crystals. A major reason for this is to compare the laboratory system with the factory operating system. Did you check to see if your laboratory system was making the same transfer of ash components as occurred in the factory?

Donovan: I've read your group's papers in this area. I don't think we have done anything quite as systematic in looking at ash as we have at color. Perhaps my co-author, John Williams, has something to add.

Williams: Naturally, what we've shown here represents the tip of the iceberg of what we've done. We have varied conditions to see if pockets in the crystal were involved, rather than just color going into crystal. None of the analyses showed evidence of a relationship of colour transfer with ash transfer – this is something that has not come up in our work yet.

Question: At what absolute pressure do you boil your sugar? Did you try different absolute pressures?

Donovan: We kept a constant temperature of 70°C throughout an experiment, which roughly corresponded to refinery pan practice.

Question: Did you look at the possibility of changing the color transfer factor by changing the molecular weight distribution of the color components? If you started with the same total color, but a different proportion of high and low molecular weight factors, did you get the same color transfer? Can you change the

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amount of color transferred to crystal by changing the molecular weight distribution of colorant?

Donovan: We didn't try that, so I can't answer that question. However, we've noticed that we don't always get the anticipated color balance when color is fractionated on membranes.

Question: The question relates to seasonal and geographic variability: do these relate to the molecular weight distribution of colorants and therefore to color transfer?

Donovan: I should think that is very likely.

Question: We have made some experiments on color transferred into crystal, growing crystals in sucrose solutions containing different colorants. We've found that caramel type colorants are more prone than others to go into crystal. Do you have any comments?

Donovan: The caramels that can be purchased have a high color transfer rate - these are the purchased caramel colorants rather than those generated in sugar processing.

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ION-EXCHANGE RESINS AND SUGAR LIQUOR DECOLOURIZATION BY ION
EXCHANGE RESINS

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ABSTRACT

Sugar colourants are removed by styrenic strong base ion-exchange
resins mainly through two mechanisms: ionic bond formation between
the anionic colourants and the active charged groups, and through
hydrophobic interaction between the hydrophobic part of colourants
and the resin matrix. The influence of these two mechanisms on
sugar liquors decolourization is studied using interfering inor-
ganic and organic compounds mixed with sugar solutions.

The competitiveness between these compounds and sugar colourants
for the resin sorption sites can elucidate the chemical nature of
colourants and the sorption mechanism involved. Resins regenera-
tion with sodium chloride is also studied and tentatively opti-
mised.

INTRODUCTION

Ion exchange resins have been used for sugar liquor decolourization
for some decades. The effectiveness of the system has been imposed
in relation to the traditional and classic systems used previously.

A better understanding of the physical and chemical inter-action
mechanisms between resins and sugar colourants can result in an
improvement of the operating conditions during decolourization and
regeneration processes.

It is known that colourants are fixed to strong base anionic
styrenic resins, mainly by chemical bond mechanisms between the
anionic colourants and the quaternary ammonium active groups, and
through hydrophobic inter-action between the colourants apolar
parts and the hydrophobic resin matrix. These mechanisms can act
simultaneously in the same colourant in an amplified ionic binding
(10).

In this work we studied sugar liquor decolourization by ion-
exchange resins in presence of increasing quantities of different
inorganic salts. The behaviour of different adsorption mechanisms

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can be evaluated. Some colourants can switch from ionic bond mechanisms to surface sorbing mechanisms which increase the difficulty of their removal from resins. The colourants fixed by surface adsorption to styrenic resins at different pH conditions were studied using HPLC analysis. The desorption of sorbic acid from styrenic resins in presence of NaCl can elucidate the anionic colourants behaviour at resin wash during regeneration.

Experimental procedures

a) Decolourization of sugar solutions containing inorganic
   compounds.

Using the technique described previously (2) a styrenic resin, IRA 900C in the chloride form, two acrylic resins IRA 458 and IRA 958 in the chloride form and an adsorbent resin, XAD-2, were contacted with a solution of cane sugar refinery carbonated liquor containing increasing quantities of sodium iodide, sodium nitrate and sodium chloride. A blank sugar solution with the same quantity of the inorganic compound without resin was used as reference solution. Decolourization was evaluated measuring Attenuances at 420 nm and pH 9.0 of each solution in relation to the equivalent reference solution. Results are presented at Figures 1, 2, 3, 4 and 5.

b) Adsorption of sugar colourants into ion-exchange resins at
   different pH conditions.

To 100 ml of sugar solution (60 ml of carbonated liquor and 40 ml of distilled water) was added a quantity of NaI to 1.5 N previously dissolved in the water portion. The solution pH was corrected to pH $x1 \pm 0.05$ with HCl or NaOH. This solution was then mixed with 10 g of air dried strong base anionic resin (Amberlite IRA 900C) in the chloride form in a closed vessel with pH and temperature sondes. Nitrogen was bubbled in the solution during 5 minutes. The mixture was agitated with a magnetic stirrer and maintained at 40ºC in a water bath during 2 hours. During this time the pH was maintained constant by adding HCl or NaOH. After two hours the resin was separated from the solution by vacuum filtration through 0.8 um Sartorius filter. A quantity of NaI equivalent to 0.2 N was added and dissolved to the filtered solution. The pH was then corrected to pH = $x2 \pm 0.05$ ($x2 < x1$) with HCl and NaOH. This solution was then mixed with 10 g of a fresh portion of IRA 900C resin, in the chloride form, as described. After two hours of contact, the resin was separated by vacuum filtration and washed with distilled water. The separated resin was then contacted in a closed flask with 200 ml of isopropyl alcohol in water (50:50) with agitation and heating at 40ºC for four hours. The solution was then separated from the resin by vacuum filtration. Samples were

kept cooled in closed flasks and were sent to IBET for HPLC analysis (Appendix).

c) Desorption of sorbic acid fixed into ion exchange resins in presence of NaCl 4M.

Styrenic resin, IRA 900C in the chloride form, was charged with sorbic acid at pH 11.0 or pH 2.0 in presence of NaCl 4M as described earlier (2). The resin was placed in a 10 ml Pharmacie column and washed with water at the same pH as the salt. A second elution was performed with a solution of NaCl 2 M and Ethyl Alcohol (30% v/v). The same test was then performed with a cationic resin, IR-120, at the sodium form at pH 11.0. Results are presented at Figures 10, 11 and 12.

APPENDIX

HPLC Analysis were performed at IBET - Instituto de Biologia Experimental e Tecnológica (Apartado 12 - 2780 Oeiras - Portugal).

Samples received from RAR were concentrated by vacuum rota evaporator at 35-40ºC from 25 ml to circa 1 ml.

Equipment: LKB-Bromma 2150 pumps (2)
            LKB-Bromma 2152 LC processor
            Shimadzu SPD-M6A Diode detector
            100 ul loop
            Ultropac Lichrosorb RP-18, 7 um, 4x30 mm pre-column
            Ultropac Lichrosorb RP-18, 5 um, 4x250 mm column

Reagents: Methanol Lichrosolv Merck
           Acetic Acid p.a. Merck
           Iso-propylic Alcohol p.a. Merck
           Distilled and dionised water at IBET

Experimental conditions:

           Eluent A: Water:Methanol:Acetic Acid (90:5:5)
           Eluent B: Water:Methanol:Acetic Acid (5:90:5)

           Programming:

        8 minutes at 1.5 ml/min 0 to 1% Eluent B
     60 minutes at 1.5 ml/min 1 to 50% Eluent B
      2 minutes at 1.5 ml/min 0%   Eluent B

References: Paton et al, 1985 (8)

The work was directed by Prof. Luis Vilas-Boas and assisted by
Dra. Maria do Rosário Bronze and Eng. Ana Luisa Simplício.

RESULTS AND DISCUSSION

a) Decolourization of sugar solutions containing inorganic
   compounds.

Sugar colourants comprise a great variety of organic compounds
being mainly anionic in alkaline conditions.   Some of these
colourants possess an amphiphilic character with hydrophobic and
polar parts.   Due to their chemical nature these compounds can be
fixed to strong base anionic styrenic resins both by ionic
mechanisms and/or hydrophobic inter-actions. Anionic colourants at
high pH are in an ionic negative form what facilitates the ionic
bond.   In the same conditions, as described earlier, carbonated
liquor of a cane sugar refinery was decolourized in presence of
increasing quantities of different inorganic salts.   It was
observed that at pH 9.0 decolourization decreases drastically as
the salt concentration increases, attaining a steady value at high
salt concentrations. The initial decreasing of decolourization can
be explained by the inorganic anions that will compete with sugar
colourants for the positive active groups of the resin.   At high
salt concentration colourants will be mainly fixed by adsorption
mechanisms to the resin surface.   Decolourization curves of
carbonated liquor with IRA 900C resin, in presence of NaI, NaNO3
and NaCl are presented at Figures 1 and 2.

It was observed that with styrenic resins the salts used have a
different interference power on decolourization at pH 9.0 (Figure
2).   Sodium iodide presents a higher interference followed by
sodium nitrate and sodium chloride.   This can be related with the
different hydration degrees of these anions ($I^- = 0$; $NO_3^- = 1$;
$Cl^- = 3$) (7).

By decreasing the liquor pH, anionic colourants will be less
ionised and decolourization decreases.   Amphoteric colourants can
also change to a positive form and will not be fixed to the anionic
resins.   At these acidic conditions the interference of the
inorganic salts, at high concentration, was lower than under
alkaline conditions (Figure 1).

The possibility that some amphiphilic colourants switch from an
ionic bond mechanism to a hydrophobic fixed mechanism, can explain
this behaviour (2). A similar behaviour was described by Yoshida et
al. (11) using BSA (Bovine serum albumine) fixed to a strong base
dextran-type ion-exchange resin.   These authors refer to a change
of sorption mechanism from electrostactic attraction between the

negative BSA molecule and the resin positive fixed ions at a pH higher than 5.05 to a hidrophobic inter-action mechanism at a pH 4.8, when BSA molecule is almost neutral.

Decolourization of carbonated liquor was also performed with anionic acrylic resins, IRA 458 and IRA 958, in presence of inorganic salts at pH 9.0. It was observed that decolourization decreases practically to zero when salt concentration increases in solution (Figures 4 and 5). The low hydrophobicity of the acrylic resin matrix resulting in a weaker hydrophobic inter-action with sugar colourants can explain this behaviour.

The comparison between the behaviour of decolourization of styrenic resins and acrylic resins suggests that the difference on interfering capacity of NaCl and NaNO3 and NaI will be related to the adsorptiom mechanisms and not to the ion-exchange mechanisms. The sorption of organic ions by low capacity ion-exchange resins have been studied by different authors (1,4). These authors explain the adsorption of organic ions onto styrenic-divinylbenzene macroporous resins through the electrical double layer theory of Stern-Gouy-Chapman. According to this theory there are two principal mechanisms on organic ions sorption to the resins: ion-exchange of the organic ion by other ion in the diffuse layer and adsorption mechanism of the organic ion on the surface of the resin. This last mechanism is strongly dependent on the electrical potential of the surface but ion-exchange is independent of this potential. The theoretical curve representing the sum of the influences on the desorption due to ion-exchange mechanism and to surface adsorption mechanism with increasing ionic strength solutions is similar to that observed with sugar decolourization with styrenic anionic resins. The theoretical curve referred to the ion-exchange mechanism is similar to the one obtained with acrylic resins.

Afrashtehfar and Cantwell (1) used p-nitrobenzenesulfonate, NBS-, as organic anion at trace concentrations to be adsorbed at a low capacity anion-exchange resin prepared by covalently fixing ammonium quaternary ions to XAD-2 resin. These authors study the adsorption of this ion in presence of increasing quantities of NaCl and NaClO4. A higher interference was observed with NaClO4 than with NaCl. This behaviour is similar to the behaviour of NaI and NaCl interfering with the sugar colourants adsorption. The ion ClO4- presents a similar ion-exchange selectivity (6) and has a low hydration capacity as iodide ion.

The sorption of sugar colourants into an adsorption resin, XAD-2, in presence of increasing quantities of inorganic salts was also studied (Figure 5). It was observed an increase of adsorption as NaCl concentration increases. This effect is referred to as salting-out effect (5,6). When NaI was used this increasing effect was not observed. The behaviour of these salts on adsorption

mechanisms can explain the differences on decolourization observed when styrenic anionic resins were used.

The same behaviour was observed by other authors (1) using low concentrations of an organic anion, NBS-, in presence of increasing concentrations of NaCl and NaClO4, using XAD-2 resin. The behaviour of NaClO4 interfering with NBS- is similar as for the iodide ion interfering with the sugar colourants.

The results observed previously can be useful to perform a more effective ion-exchange regeneration. Salt is a cheap chemical but presents a lower interference with some colourants adsorbed to the resins. When resin regeneration is performed at low salt concentration low charged colourants are released from the resin (3). At this low ionic strength a low pH can facilitate the colourants removal (Figure 6).

As the salt concentration increases, during regeneration, colourants with a high anionic charge will be desorbed from the resin. An increase of salt alkalinity will increase the colourants desorption from the resin by changing amphiphilic anionic colourants to a ionic form that can be displaced by the chloride ions in solution.

In the last part of the regeneration, salt is washed out of the resin column and the ionic strength decreases again. Sugar colourants fixed at the resin surface are released as the salting-out effect decreases. As the influence of ion-exchange increases at low ionic strength these colourants can be maintained ionically fixed to the resin. This can decrease the resin capacity and lower the regeneration efficiency. To avoid this effect an acid wash can be made after salt regeneration. At a low pH part of the colourants will be at a neutral form and will not be maintained in the resin.

This acid wash was tested in a one liter column with a styrenic divinyl benzene anionic resin, IRA 900C, in the chloride form. After charging the resin with 50 BV of carbonated liquor it was regenerated with 3 BV of NaCl at 100 g/l followed by an acid regeneration at low salt concentration (1 BV of NaCl at 30 g/l mixed with 0.5% of HCl). Results are presented at Figure 7.

b) Surface adsorption of sugar colourants into ion-exchange resins at different pH conditions.

The previous results show that some anionic amphiphilic colourants are difficult to be removed from anionic styrenic resins with a normal salt regeneration. These colourants can be fixed to a styrenic resin in presence of a high salt concentration and changing the pH conditions.

As described in Experimental Procedures colourants were fixed to a anionic styrenic resin, IRA 900C, in the chloride form, in presence of NaI 1.5 N. Under these conditions the resin will adsorb colourants mainly by surface hydrophobic inter-action mechanisms. The resin was then removed and the solution pH was lowered. This solution was then contacted with fresh styrenic resin. A fraction of colourants will change to a neutral form or to a low anionic charge and will be fixed to the fresh resin in contact with the solution through surface adsorption mechanisms. These colourants were then removed from the resin, after washing with distilled water, with a solution of iso-propyl alcohol in water (50:50).

Tests were performed at 0.5 pH intervals from pH 9.0 to pH 2.0. HPLC profile of sample PH8075 (pH drop from 8.0 to 7.5) and PH3530 (pH drop from 3.5 to 3.0) at 313 nm are presented at Figures 8 and 9. This wavelength is a good compromise to detect simultaneously phenolics and flavonoids (9).

Further studies are necessary for an identification of these compounds.

c) Desorption of sorbic acid fixed into ion exchange resins in presence of high concentrations of NaCl.

The desorption of sugar colourants fixed into anionic styrenic resin IRA 900C when ionic strength decreases was studied substitut-ing the sugar colourants by an organic anion with amphiphilic character. Sorbic acid CH3-CH=CH-CH=CH-COOH was used. Sorbic acid was analysed by UV/VIS GBC 916 Spectrophotometer at 260 nm.

In these tests sorbic acid was fixed to IRA 900C in the chloride form, and IR 120 in the sodium form, in presence of NaCl 4M at pH 11.0 and pH 2.0. Excess of sorbic acid was removed by washing the resin with NaCl 4M at the same pH (Experimental Procedures 2.b).

Desorption was performed with water alkalinised at pH 11.0 with NaOH or acidified to pH 2.0 with HCl. A second desorption was made with a solution of NaCl 2M with Ethyl Alcohol 30% at the same pH.

During the water wash, at acid conditions, sorbic acid was desorbed from the anionic resin (Figure 10). At a high pH, with the sorbic acid in the ionised form, no desorption was observed from the anionic resin (Figure 11). This can be explained by the positive charge of the quaternary ammonium cations fixed covalently to the styrenic di-vinyl-benzenic matrix. This positive charge must impede the desorption of sorbic acid, charged negatively. The same effect is not observed when the resin is charged negatively as in the cationic resin (Figure 12).

These results can explain the extra colourants removal when acidic wash is performed (Figure 7).

CONCLUSION

Sugar colourants comprise a variety of organic compounds of different chemical nature. These compounds can be adsorbed into styrenic anionic resins mainly by ion-exchange or hydrophobic inter-action mechanisms. By varying pH of the solution, some colourants can switch from one to another sorption mechanism. The presence of increasing quantities of NaCl increases the colourants surface adsorption on styrenic resins. Desorption of colourants from resins can be enhanced by decreasing the salt pH at low ionic strength and by increasing it at high salt concentrations.

ACKNOWLEDGEMENT

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Figure 1
Decolourization of Carbonated Liquor
with IRA900C in presence of NaI
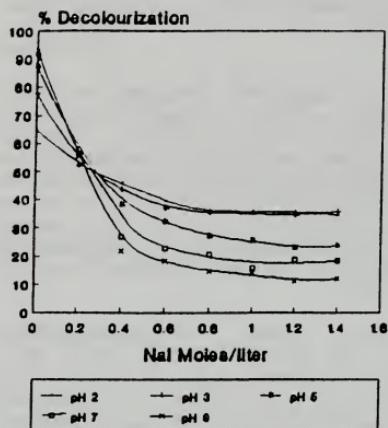
Figure 2
Decolourization with IRA900C at pH 9
in presence of inorganic compounds
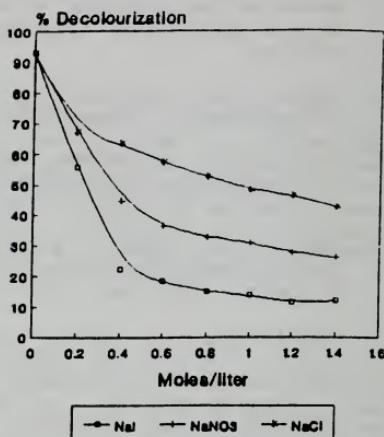
Figure 3
Decolourization with IRA458 at pH 9
in presence of inorganic compounds
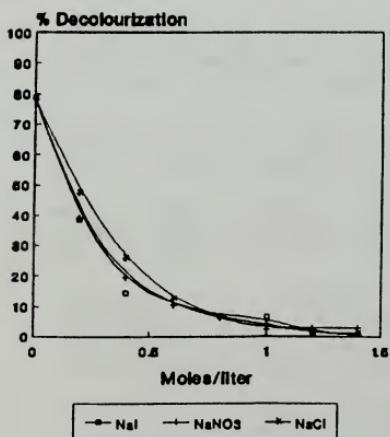
Figure 4
decolourization with IRA958 at pH 9
in presence of inorganic compounds
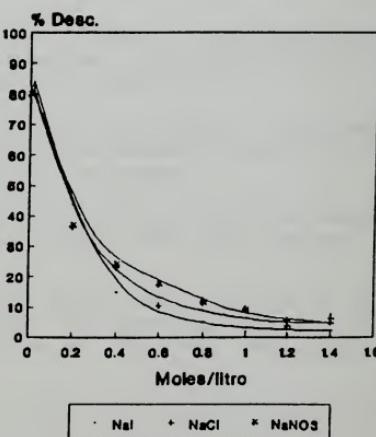
Figure 5
Decolourization with XAD2 resin
In presence of inorganic compounds
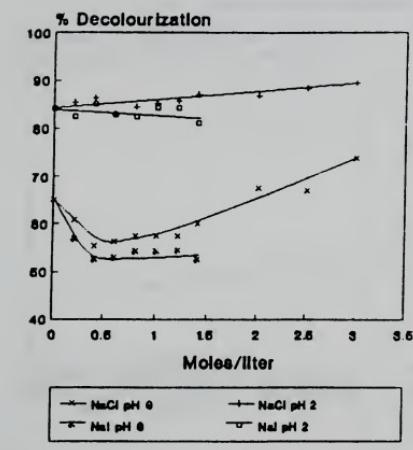
Figure 6
Influence of pH on Ionic Bond and
Hydrophobic Action during Regeneration
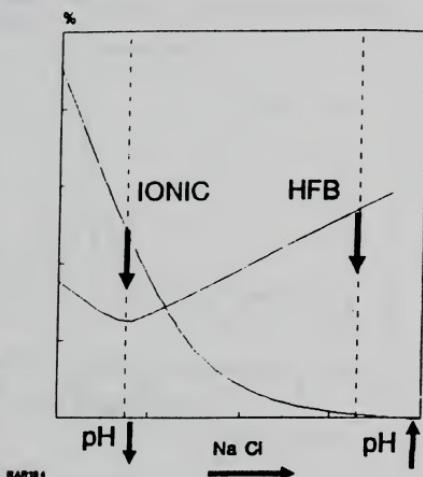
Figure 7
Regeneration with 3 BV NaCl
and 1 BV NaCl + HCl
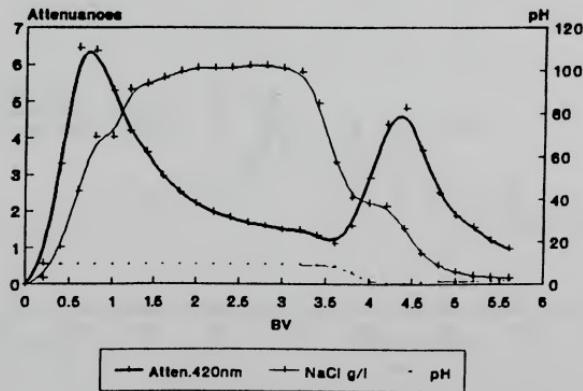
Figure 8
HPLC profile of sample PH8075

=SPD-M6A POST ANALYSIS multi-chromatogram
CH1: 313-313(nm)
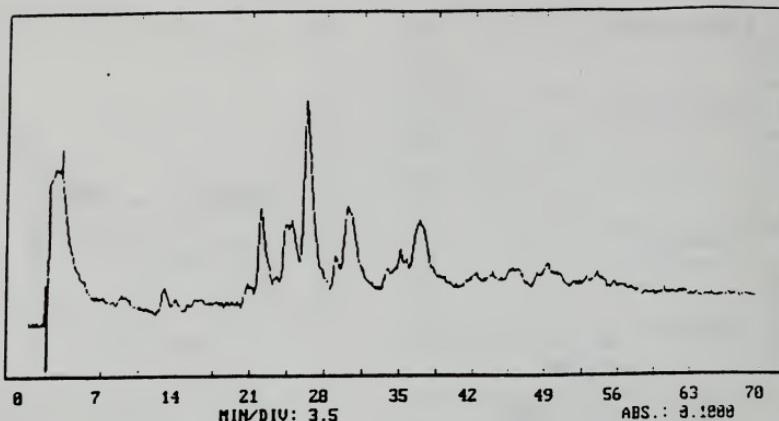
Figure 9
HPLC profile of sample PH3530

=SPD-M6A POST ANALYSIS multi-chromatogram
CH1: 313-313(nm)
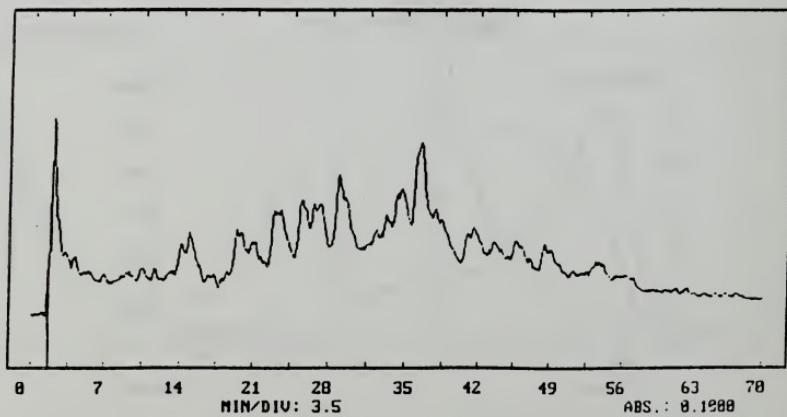
Figure 10
Desorption of Sorbic Acid from IRA900C
with NaCl pH2 / Water pH2 / NaCl+EtOH
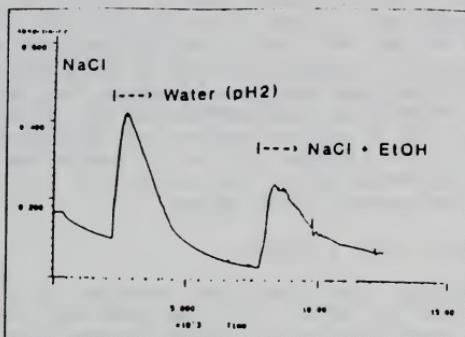
Figure 11
Desorption of Sorbic Acid from IRA900C
with NaCl pH11 / Water pH11 / NaCl+EtOH
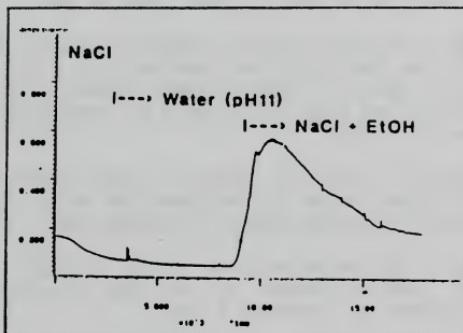
Figure 12
Desorption of Sorbic Acid from IR120
with NaCl pH11 / Water pH11 / NaCl+EtOH
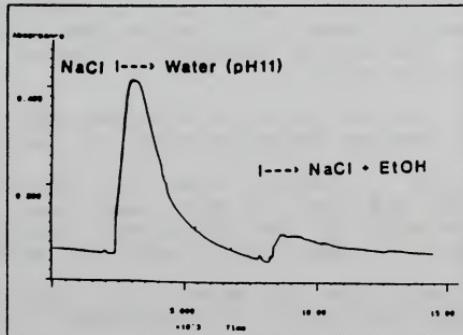
DISCUSSION

Question: Congratulations on your fine work. I especially liked the ingenious way you changed the pH to selectively take off colorants. An observation on the differences in amounts of color taken off by sodium iodide with changing pH: if you plot absorbance (or color) against pH, you'll match the amounts of color taken off at different pH's. It's a matter of de-ionization of colors, as you indicated in the paper.

Bento: Yes, I agree.

Question: One question: looking at the spectrum of fraction 26 minutes (by HPLC), it's not possible to see much absorbance at 400 nm. Have you tried adding up the contributions at visible color wavelengths to see if they match the color measured?

Bento: We need larger samples to do that, because at these low dilutions the visible absorbance is not measurable because of the noise. We will use higher concentrations in future work. We concentrated from 25 ml to 1 ml: perhaps we should concentrate from 100 ml to 1 ml.

Question: Did you look at any regeneration using 0.1% or 0.2% sodium hydroxide in conjunction with the 10% sodium chloride, to determine regeneration efficiencies?

Bento: To increase the pH up to 12? No, we didn't, because we found precipitation if pH was increased above 11 or so. We maintained pH between 2 and 9.

Question: It's always been our recommendation to add a little caustic to brine to get that "one-two" punch. The caustic puts the carboxylic groups in the sodium form - the most soluble - while the brine (the strong electrolyte) removes the color that is hydrophobically bound.

Bento: We are working with carbonated liquors with high calcium. If we increase the pH too much, we find that we get precipitation.

Question: A comment: we've found the past work of John Williams, at Tate and Lyle, in this same area, to be of great interest, particularly in the differences shown in acrylic vs styrenic resins, with high and low molecular weight colorants.

Luis Bento has not shown that there is a difference between styrenic and acrylic as a dependence on pH.

I look forward to future work explaining the reason for this difference.

<u>Bento:</u> In a previous paper (SIT 1992) we used IR458 acrylic resin; in this paper, we used IR958, a macroreticular resin. In fact, we noted a small increase in decolorization - the 958 can have a little more absorption than the 458.

With acrylic resins, we made the experiments only at high pH as an instability was observed at low pH.

EFFECT OF MAGNETIC FIELD ON EVAPORATOR SCALE

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ABSTRACT

The causes and problems of scale formation in sugar factory
evaporators are outlined. The installation of permanent magnets in
a set of evaporators and the effects of the magnetic field and
induced electrical field are described.

Results of the magnetic field treatment on reduction and control of
evaporator scale, and on the nature of scale, are presented. Cost
savings from reduction in scale formation, evaporator cleaning and
shutdown time are discussed.

INTRODUCTION

A pivotal point in the manufacture of raw cane sugar is evaporation
of juice to syrup. Evaporator efficiency, and frequency of
shutdown for evaporator clean out, are major cost factors in
operation.

Formation of scale in sugarcane juice evaporators is a fact of
life. This scale causes many problems:

1) Reduced heat exchange and reduced efficiency of the
   evaporators.

2) Lowering of throughput resulting in added expense in
   operation.

3) Lengthy cleaning periods during which evaporators must be
   shut down.

4) Cost of chemicals and labor involved in cleaning.

5) Lost time and reduced cane throughput while evaporators
   are being cleaned.

THEORY

Several theories have been advanced on the affects of magnetic treatment devices; these have recently been summarized by Abernethy et al (1). However, there appears to be no discrimination between electromagnets and permanent magnets in the theories mentioned. In the case at hand, only permanent magnets are considered.

When a jet of conducting fluid with velocity moves through a magnetic field flux at right angles to the field's origin, an electron excitation occurs in the conductive fluid. The conductivity is related to the dissolved solids in the fluid. The ions of the suspended solids are either negatively or positively charged, and it is their coming together to form neutral particles that causes precipitation. The magnetic field induces an electrical field, which causes the charged particles to move more quickly. The increase in speed of the charged ions causes increased rate of formation of very small neutral particles. The small individual neutral particles do not form the usual large clusters of precipitate known as "scale", but remain moving through solution, eventually settling out as loosely bound clumps of precipitate, when solution density i.e. Brix increases and particle movement in slowed down.

TREATMENT

M. A. Patout Sugar Mill has four sets of evaporators: one large set of triples which has a heating surface of 43,000 square feet, one set of quads which has 28,000 square feet and two smaller sets of triples which have a combined heating surface of 36,000 square feet. The large triples and the quads are the two sets which work 95% of the time. The two small sets work when the larger ones are being cleaned. The average time for cleaning the evaporators is 12 to 14 hours resulting in a loss of grinding capacity of some 2000 ton of cane a day.

When this magnetic treatment was first introduced, the senior author was very skeptical, but as the offer was made on a no result-no cost basis there was little cost involved in installing the units. Prior to the installation, M. Walet of Hydrodynamics Corp. discussed with staff at M. A. Patout the various densities involved and the gallon throughput of juice and syrup, and sized the magnets accordingly.

Before grinding, the units were installed on the big set of triple evaporators. The units consist of three stainless steel spools, flanged on both ends for installation and removal, containing a number of steel rods which are permanent magnets. The size of these spools was increased according to the body on which they were

working: 10" for the 1st body; 12" for the second and 14" for the third evaporator body.

Prior to grinding, the evaporators were boiled out with caustic soda and then with a solution of hydrochloric acid. The tubes were examined for cleanliness and lack of scale. When grinding started the evaporators were monitored for performance.

In the first body of the evaporators there has been installed a pilot evaporator which contains the same size tubes as the body. In the third set there is another pilot, with a heating element; the electricity needed to maintain this element at a constant temperature is measured. As the element is coated with scale the demand for electricity drops. These installations have been in place for three years now and were installed by S. Clarke of Audubon Sugar Institute, in a separate project on scale formation in evaporators.

OPERATION: OBSERVATIONS AND DISCUSSION

For many years now (at least 14) it has been found that after about 80,000 tons of cane have been passed through the mill the evaporators need cleaning. This has been so established at M. A. Patout factory that boilout was regularly programmed on this basis. This year (1991), however, after 80,000 tons the evaporators still seemed clean and were working with practically the same efficiency as at the start of crop. Nevertheless, they were shut down, rinsed out with water and examined.

RESULTS AND DISCUSSION

The first and second bodies were very clean, but the third had some scale present. We decided to go ahead and clean the bodies as they were shut down. The evaporators were treated as usual, then inspected and put back into service. The evaporators were then allowed to go until 120,000 tons of cane were ground, and were inspected again. The same results were noted. The evaporators were treated and put back into service.

We finished up grinding allowing 160,000 tons of cane to pass through before evaporators were taken off line for treatment. At the end of grinding the evaporators were rinsed out with water only. During the off-crop season when the evaporators were cold and dry, the two pilot evaporators were taken out and the scale was analyzed. The tubes were also treated to clean them.

On visual examination the first two bodies were still very clean. The third body had some scale in it but of an unusually soft type. The normal scale in the third body is usually very hard to remove

and has a very hard surface. The scale in this case, however, was very soft and fluffy in appearance and easily removed.

The tubes were immersed in a 3% hydrochloric acid (HCl) solution for a period of time to clean them. The scale was not dissolved but fell off the tubes leaving them clean and smooth. The scale was weighed on some other tubes and, in comparison to previous years, was about 60% less. It was noted that the electrical demand for heat lasted longer than previous years, again indicating a slower build-up of scale and better maintenance of heat transfer and evaporator efficiency. No physical differences have been noticed in the sugar product quality made at M. A. Patout nor have we received any customer complaints. The ash level in sugar product did not increase.

Next year, before grinding starts, the evaporators will be boiled only with HCl and then checked. If it is possible to clean only with HCl, savings will result of about 10-12 hours in downtime per washout, as the majority of time lost is because of caustic soda treatment, in addition to savings in caustic soda cost. M.A. Patout plans to install an additional set of magnets on the pre-evaporator for the 1992 season.

Last year we saved about 60% of the cost of caustic soda usage as the amount of soda used dropped from 0.06 gallon/ton to 0.02 gallon/ton. Caustic soda costs about $1 per gallon; with a grinding rate of 1,000,000 tons this equates to $40,000 a year. Next year may be even better, if caustic use can be eliminated. With the cost of the units being $60,000, after two years we will be saving money.

ACKNOWLEDGEMENTS

REFERENCES

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      tor scale inhibition evaluation. Proc. Austr. Soc. Sugar Cane
      Technol. pp. 220-226.

DISCUSSION

Question: Can you tell us something about the effect of magnetic field on the nature of the scale?

Clarke: I can only tell you about the experience at M.A,. Patout, as described in the paper. Unlike the usual hard, firmly attached scale, this was a loose crumbly scale, that could be removed with acid treatment only; the usual caustic treatment was not required to remove this scale. We don't have any information on the composition - just on the ease of removal, and increase in time between scale removals.

Question: Does the scale go to molasses or into the sugar?

Clarke: Apparently to molasses; there was no increase observed in ash in raw sugar, and no customer complaints. More measurements are planned for the next crop.

Question: With regard to the savings in chemicals: you mentioned savings in caustic, but not any savings in acid. If the boil out time were increased 50% to 100%. There would be considerable savings in muriatic acid that was not used.

Clarke: That's a good point. Less acid was used, because boil-outs were less frequent. I believe that saving was omitted from the paper's calculations, because of the relatively higher cost of the caustic.

Question: Can you comment on why electromagnets don't work, and if magnets are installed right in the pipes. If that is the case, the flow rate would be increased and scale would have less chance to form on the pipes.

Clarke: I believe the throughput volume is the same with and without the magnets. The magnets are installed on the input line.

As to the electromagnets: I can only quote literature references and personal communications: they don't work to decrease scale: The permanent magnet is necessary - presumably because the fluctuations in the electromagnets do not create a fixed electrical field.

Chairman: Elimination of the use of sodium hydroxide in evaporators has a great deal of importance to us, because the liquid waste goes to ponds and ultimately to ground water. This water has to meet drinking water standards. The sodium hydroxide used in evaporator cleaning raises this sodium level considerably -it would be very valuable to get rid of that.

DETERMINATION OF ANIONS AND CATIONS IN SUGAR FACTORY SAMPLES BY ION
CHROMATOGRAPHY

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ABSTRACT

Ion chromatography (IC) offers the opportunity to determine
routinely anions and cations in aqueous solutions of different
origin either by isocratic or gradient separation of the analytes.

We have investigated both anion and cation composition of factory
juices, e.g. raw juice, thick juice, molasses, as well as white
sugar.

For that purpose we developed simple isocratic IC separations using
AS4A, AS9 and CS10 columns from Dionex. Simultaneous determination
of 8 anions and 5 cations, respectively, appeared to be possible.

Special attention has been given to the isocratic separation of
sulphite, malate and sulphate.

Comparison of the IC results with conventional methods for analysis
of individual anions and cations reveals good correlations.

In conclusion, using IC, more information on the anion/cation
composition of sugar factory samples can now be obtained in less
time than with previously available methods.

INTRODUCTION

The use of high performance liquid chromatography (HPLC) in sugar
industry laboratories for the analysis of organic acids and
carbohydrates is now daily practice (1,5,6,9,12,15,20).   The
commonly used type of columns contain strong cation exchange
resins. These resins are applied in either hydrogen, calcium,
sodium or potassium form, depending on the chromatographic
separation needed.

For special applications, as for instance separation of oligo-
saccharides or disaccharides, columns are available in other ionic
forms, e.g. lead and silver, and in different percentages of cross-
linking of the polystyrene-divinylbenzene resin.

Recent improvement of the quality of anion exchange columns and the concurrent development of pulsed amperometric detection at present offer an alternative system to separate and measure carbohydrates in sugar products (12-15,18,20). The rise of ion chromatography (IC) among other settled liquid chromatographic methods has opened a new field of applications for the analytical chemist. In addition to the mentioned analysis of carbohydrates should be added the determination of anions and cations by IC.

In the 1980's, reversed-phase ion-pair chromatography (RP-IPC) using spectrophotometric detection seemed to be the chromatographic solution for the analysis of inorganic anions (3,11). However, the necessary optimization for convenient and reliable separation of anions in different samples appeared to be cumbersome , as was confirmed by our first trials. Furthermore, the presence of so-called "system peaks" in the chromatograms, the relatively long equilibration time of the chromatographic system, and the short column lifetime may be considered 'disadvantages' of RP-IPC.

In the late 1980's, some new types of anion exchange resins became available which did not have the disadvantages observed with RP-IPC, while enabling comparable and even better separations of anions. Ever since, the key to success in chromatographic measurement of anions is the choice of the right IC column. Only when a special problem is encountered (an example will be discussed in this paper) special attention must be given to method development, i.e. combination of column(s), composition of mobile phase and isocratic or gradient elution. Several examples of successful application of IC in determination of inorganic anions in sugar factory samples are already reported in literature (4,8,14,16,21). Until the present, IC of mono- and divalent cations required column switching or gradient step changes to determine several cations in one chromatographic run. Fortunately, recent development of moderate capacity, high efficiency, cation exchange columns by Dionex now allows an isocratic separation of mono- and divalent cations without column switching.

This paper deals with the application of IC methods for the determination of different anions and cations present in sugar factory samples. There are several reasons why we have put effort into developing IC methods:

- IC can be applied to a variety of sugar factory process problems and research items as well as to the quality control of products (4,7,8,14,16,19,21,22).

- As chromatography has the potential of the analysis of different components in the same run, results may be obtained much faster by IC than in the case where each component has to be analyzed individually by a conventional method.

- Development of simple, reliable and fast IC methods which are available for application - a major goal of this work.

In this paper several examples will be presented of IC separation and measurement of anions and cations in sugar factory samples. Special attention has been given to the separation of sulfite, malate and sulfate. The relation between IC results and data obtained by conventional methods will be discussed.

MATERIALS AND METHODS

The IC's of anions and cations were conducted on QIC and DX-100 Dionex chromatographic systems, respectively. Both systems were equipped with conductivity detector. Samples were introduced with a 50 μl and a 25 μl loop for IC of anions and cations, respectively. Prior to injection, samples were diluted with "Milli-Q-water" (Millipore), if necessary, and those containing insoluble matter were filtered through a 0.45 μm membrane filter.

In the QIC system an IonPac AS4A and/or AS9 column was used at ambient temperature for the separation and measurement of anions. The analytical columns were preceded always by either an AG4A or a AG9 guard column. Between analytical column and detector, an AMMS (anion micromembrane suppressor) was installed using 12.5 mM sulfuric acid at 3 ml/min. as regenerant. The mobile phase consisted of a mixture of Na_2CO_3 and $NaHCO_3$ dissolved in Milli-Q-water. The desired composition and flow rate of the eluant were defined by the required separation of anions: see Results and Discussion.

For the determination of cations the IonPac CG10 guard and CS10 analytical columns (Dionex) were used, installed in the DX-100 system. The eluant was 30 mM HCl and 6 mM DL-2,3-diaminopropionic acid monohydrochloride (DAP) in Milli-Q-water.

In order to decrease eluant conductivity, the CMMS-II cation micromembrane suppressor was applied in combination with a Dionex AutoRegen System. As regenerant, 100 mM tetrabutylammoniumhydroxide was used.

For data acquisition the four channel HP ChemStation (Hewlett Packard Corp.) was used. Calibration solutions and mobile phases were prepared using analytical grade chemicals and Milli-Q-water. For the stabilization of sulfite in a calibration solution it was necessary to take special precautions as will be discussed in Results and Discussion.

RESULTS AND DISCUSSION

IC of anions

The AS4A and AS9 columns (Dionex Corp.) are anion exchange resins designed for the separation of inorganic anions. These columns have the potential to separate a few organic anions, e.g. formate, acetate, malate and oxalate.

Using the recommended eluant buffers, i.e. 1.80 mM Na_2CO_3 and 1.70 mM $NaHCO_3$ for the AS4A column, and 2.00 mM Na_2CO_3 and 0.75 mM $NaHCO_3$ for the AS9 column, at a flow rate of 2.0 ml/min., and chromatograms of standard anions are obtained as shown in Figure 1 and 2. It can be seen that most relevant anions are well separated. However, if sulfite, malate and sulfate are present in the same sample neither the AS4A nor the AS9 column can determine all three analytes.

After several attempts to optimize the mobile phase composition, we did not succeed in the separation of these analytes: there always remained an unacceptable overlap between the malate and sulfate peak on the AS4A column and between sulfite and malate on the AS9 column.

The IC determination of sulfite is of particular interest since the widely used Monier-Williams method for sulfite is very cumbersome and time consuming. Sullivan and Smith (17) and Anderson et al. (2) reported a modification of the Monier-Williams method in which the trapped sulfite after distillation is determined by IC. To our knowledge, the AS4A column can replace the Monier-Williams method completely in many applications. Sulfite data comparable to Monier-Williams can be obtained for samples (raw juice, thin and thick juice) in a much shorter analysis time: i.e. 10 min instead of 2-3 h. In addition accurate IC data for chloride, nitrite, nitrate, phosphate and oxalate are obtained. In sulfate but not sulfite or malate, is of interest, the use of the AS9 column is recommended.

As analytical and research chemists we were determined to develop an IC separation of all anions in one (isocratic) run. A closer look at the chromatographic separation on the AS4A and the AS9 column, indicated that both columns in series, theoretically, could of sulfite, malate and sulfate. After optimization of the mobile phase composition (1.8 mM Na_2CO_3 and 0.8 mM $NaHCO_3$), the separation of the 8 anions was achieved as shown in Figure 3.

It should be noted that, because of the high backpressure with both columns in series, it was necessary to decrease the eluant flow rate to 1.3 ml/min. to keep the back-pressure below the pressure

limit (approximately 1900 psi) of the pump installed in the QIC system.

Examples of the application of the AS4A and AS9 columns in series on a raw juice and a thick juice sample are shown in Figure 4 and 5, respectively.

Figures 3,4 and 5 show that the retention times of the same anions in the raw juice chromatogram are less than those found in the chromatograms of the standard and of the thick juice. The reasons are column life-time and column fouling. The runtime for the separation of all 8 anions on new AG4A, AS4A and AS9 columns in series amounts to approximately 30 min. However, after injection of several samples, retention time of the analytes rapidly drops. Apparently, some components present in sugar factory samples block part of the functionality of the anion exchange resins, causing a decrease of their capacity. After an estimated 50-100 injected samples, the total runtime is decreased to about 25 min. On further injections, the retention times decrease more slowly while the separation of the anion remains acceptable up to 500-700 injections; meanwhile the guard column has been changed only one time.

Clean-up of the separator columns (according to Dionex instructions) did not restore the anion exchange resins to their original retention capacity. Stewart (16) described a more comprehensive clean-up procedure for an AS4 column which may be applicable to the AS4A column as well. Notwithstanding the decrease in retention, separation of anions remains acceptable after several hundreds of injections, with use of only the Dionex clean-up method up to now.

The IC separation of anions as described in this paper have been used for research and trouble shooting purposes (7,22) as well as for quality control of products. Anion composition in a variety of samples, e.g.juices, sugar, lime sludge and scale, has been determined. In the analysis of anions in samples from the vacuum pan station, e.g. syrups and molasses, IC appears to give false results for sulfite. The sulfite concentration in the samples as measured by IC was several times lower than the data obtained by the Monier-Williams method. Probably IC measures only free SO_2, which apparently forms part of the total SO_2 present in these highly concentrated samples. A sample preparation step in which the SO_2 bound to organic compounds is released prior to IC analysis is under investigation.

Stabilization of sulfite in aqueous solutions

When sulfite is dissolved in water it rapidly oxidizes to sulfate as a consequence of the presence of oxygen either present in the water and/or diffused from air into the solution. Several

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scientists have recognized the problem of oxidation of sulfite and
attempted to overcome this by addition of any stabilizer. Anderson
et al. (2) proposed the use of 0.1 M NaOH/0.1% formaldehyde and
deaerated water in order to prepare sulfite standard solutions.
The 1000 ppm standard was stable for 3-5 days; 5-30 ppm standards
were stable for 1-3 days.   The efficiencies of three groups of
potential sulfite-stabilizing compounds were found to be in the
order: carbonyls > alcohols = saccharides (10). A mole ratio of 1:1
between formaldehyde and sulfite was sufficient to stabilize a
sulfite solution for at least 3 days.   The lower stabilizing
efficiencies of the alcohols and saccharides examined could be
compensated by using large excesses of these compounds.

In addition to the stabilizing effect of monosaccharides, examined
by Lindgren (10,) we investigated the sulfite-stabilizing potential
of sucrose in concentrations present in sugar factory samples.
When a 1000 ppm sulfite standard is prepared in (deaerated) water
containing 10 g/l sugar (low in SO_2) the standard is stable for at
least 4-5 days at ambient temperature.   Without this precaution,
more than 80% of the sulfite will be oxidized to sulfate within 4
days.

With respect to sugar factory samples it will be obvious that the
sulfite in these samples is automatically stabilized by the
presence of sugar.   If samples are injected in the IC system
immediately after dilution with (deaerated) water then the
oxidation of sulfite may be considered to be negligible.

IC of cations

The CS10 cation exchange column offers the possibility of isocratic
separation of mono- and divalent cations without column switching.
Mobile phase is a mixture of HCl and DAP, with eluent composition
optimized to obtain the best possible separation of analytes at a
minimum analysis time.   We found 30 mM HCl and 6 mM DAP to be the
most convenient standard eluent. An example of this IC separation
of 6 cations is shown in Figure 6, whereas Figure 7 and 8 illus-
trate the applicability of IC cation analysis in raw juice and
thick juice, respectively.   Concentrations of divalent cations in
process juice samples taken after juice purification and decalcifi-
cation are very low compared to those of sodium and potassium. So,
quantitative determination of both mono- and divalent cations in
one chromatographic run is impossible for thick juice because of
different concentration ranges. Therefore two separate injections
of the differently diluted juice sample are carried out.

Quality features of IC measurements

The use of chemical suppressor devices virtually eliminates eluant conductivity while analyte conductivity is enhanced, resulting in low detection limits and a broad linear working range of analyte concentrations.

These advantages of IC with chemically suppressed eluant conductivity are confirmed by our experience. For anions separated on the AS4A and AS9 columns in series we have found minimum detections levels in the range 0.1-2.0 ng, the lowest and highest level for chloride and oxalate, respectively. With an injection loop of 50 μl, the lowest detectable concentration of anions is therefore 2.0-40 ppb. Detection limits for cations are of the same order of magnitude, namely 0.3-2.5 ng (lowest limit for lithium and highest limit for calcium), converting to 10-100 ppb when a 25 μl sample is injected.

For linearity between a detector signal and analyte concentration we established that for anions there is a linear detector response in the concentration range 0 up to 10-15 ppm, whereas for cations the linearity extends to concentrations of 20-25 ppm. Attention must be drawn to the fact that conductivity is dependent on temperature. Because the conductivity detectors in the QIC and DX-100 chromatographic systems are neither thermostatted nor insulated, fluctuation of temperature definitely can influence detector response.

With good linearity of detector response, a one point calibration is sufficient for those ions of interest. The relative standard deviations < 0.9% as determined by Tungland (21) are in agreement with our observations of the accuracy of IC measurements. In order to estimate the accuracy of IC data, we have compared the IC results for several anions and cations with results obtained by other methods. Three weekly composite samples of thick juice, which are routinely analysed by several different "conventional" methods in our laboratory, have been analysed by our IC methods. Results of the comparisons are set out in Table 1.

At first, nitrite by IC and colorimetric measurement results were not in agreement. After thorough investigation we concluded that the cause was the colorimetric measurement: poor linearity of the colorimetric method because of insufficient dilution of the samples resulted in incorrect nitrite data. Thus, initiated by IC measurement of nitrite, the colorimetric method was improved and comparable results were obtained. Sulfite by IC and Monier-Williams are in reasonable agreement. The somewhat lower values obtained by IC may result from the fact that in thick juice a small part of sulfite is bound to organic compounds. In principle, IC

determines only the free SO$_2$; this may explain why the total SO$_2$ analysis by Monier-Williams gives higher sulfite concentrations.

The IC results for sodium, potassium and magnesium are in good with AAS analysis. Calcium gives significantly different results in comparison to IC. This phenomenon was also recognized by Stewart (16) and will be subjected to further study.

CONCLUSIONS

Ion chromatography with chemically suppressed eluent conductivity appears to be very suitable for reliable and fast determination of ions in sugar factory samples. By the right choice of column(s) and optimization of mobile phase composition, isocratic separation and measurement are possible for 8 different anions (chloride, nitrite, nitrate, phosphate, sulfite, malate, sulfate, oxalate) or 6 for different cations (lithium, sodium, ammonium, magnesium, calcium). When desired, bromide, strontium or barium can be determined by IC.

In comparison to more conventional methods for analysis of ions, e.g. colorimetry, flame photometry and atomic absorption spectrome-try, IC has several advantages:

- dilution and/or filtration is the only sample preparation required before injection

- the IC systems are stand-by, ready for use within half an hour

- the analysis time of a chromatographic run is short, maximum 10-20 min

- from 1 chromatographic run data can be obtained for several ions

- detection limits range from 0.1-2.0 ng for anions and from 0.3-2.5 ng for cations

As a consequence of the abovementioned properties IC is, and has already proven to be, a very convenient method for trouble-shooting and research purposes as well as for quality control of products.

SUMMARY

Ion chromatography (IC) offers the opportunity to determine routinely anions and cations in aqueous solutions of various origin, either by isocratic or gradient separation of the analytes.

We have investigated both anion and cation composition of factory juices, e.g. raw juice, thick juice, molasses, as well as of white sugar.

We have developed simple isocratic IC separations using AS4A, AS9 and CS10 columns from Dionex Corporation. Simultaneous determination of 8 anions and 6 cations appeared to be possible. Special attention has been given to the isocratic separation of sulfite, malate and sulfate.

Comparison of the IC results with conventional methods for analysis of individual anions and cations reveals good correlations.

In conclusion, using IC, more information on the anion/cation composition of sugar factory samples can now be obtained in less time than with previously available methods.

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Table 1. Analysis of ions in thick juice by IC in comparison to other methods

Ion	Sample	IC	Other	Method type method
NO_2	1	29	29	Colorimetry
(mg/1000 g	2	14	11	(sulfanilamide/
dry solids)	3	30	25	N-(1-napthyl)
				ethylenediamine
SO_2	1	439	470	Monier-Williams
(mg/1000 g	2	386	411	
	3	323	343	
Na^+	1	0.196	0.296	Flame
(g/100 g	2	0.171	0.174	photometry
dry solids)	3	0.274	0.281	
K^+	1	1.096	1.048	Flame
(g/100 g	2	1.302	1.244	photometry
dry solids)	3	0.962	0.952	
CaO	1	6.28	8.09	Atomic
(mg/100 g	2	7.68	9.90	absorption
dry solids)	3	9.04	10.89	spectrometry
MgO	1	2.30	2.39	Atomic
(mg/100 g	2	3.22	3.35	absorption
dry solids)	3	1.49	1.53	spectrometry

Figure 1. Standard anions on AS4A
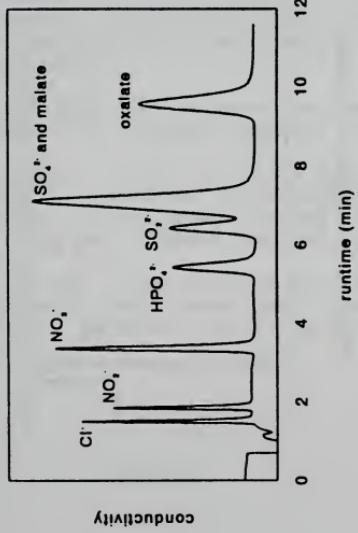
Figure 2. Standard anions on AS9
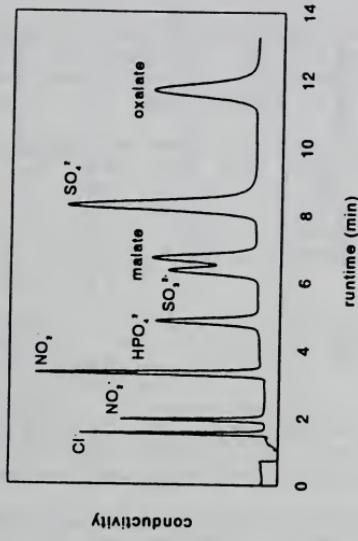
Figure 3. Standard anions on AS4A/AS9 in series
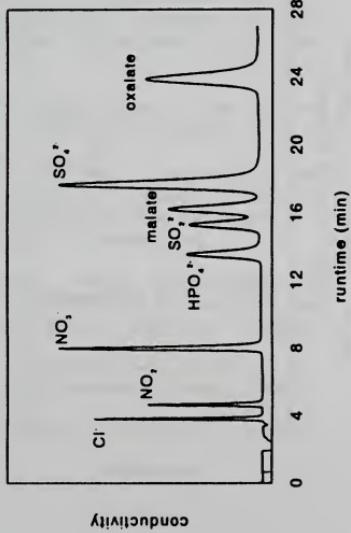
Figure 4. Chromatogram of raw juice on AS4A and AS9 in series
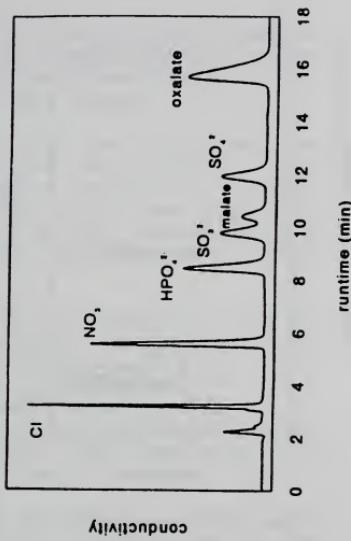
Figure 5. Chromatogram of thick juice on AS4A and AS9 in series

Figure 6. Standard cations on CS10

Figure 7. Chromatogram of raw juice on CS10

Figure 8. Chromatograms of thick juice on CS10
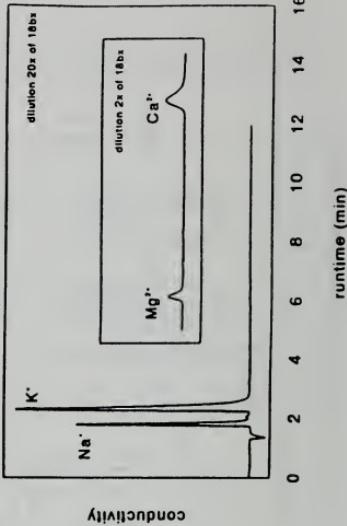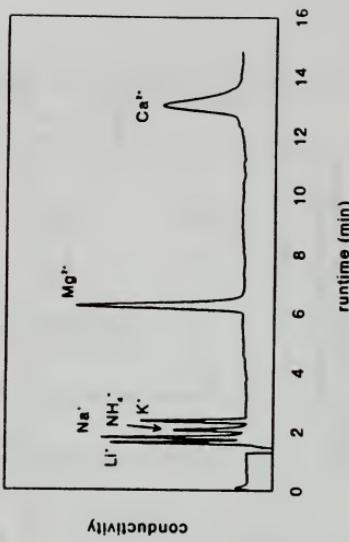
DISCUSSION

Question: What concentration of sugar solutions did you inject to detect the sulfite? Did you have to inject rather a large amount of sucrose, causing the column to deteriorate rather rapidly? I'm referring to the analysis of sulfite in white sugar.

de Bruijn: We made a concentration of 5g/100 ml for injection. We did not find that the columns deteriorated very rapidly. Of course, brand new columns show, with repeated injections, a rapid decrease in retention time, and so one may think the column is dying, but this change stabilizes after 50 or 100 injections, and then resolution remains acceptable up to 500-700 injections, in our experience.

Question: In the beet factory, one ion we can control by varying conditions is citrate. You did not mention citrate - did you analyze that?

de Bruijn: In our experience, that analysis is not possible with this system. The reason may be that citrate, a tricarboxylic acid, is adsorbed strongly to the column during the analysis, and therefore not separated in this analysis time.

Question: We know that in decolorization of syrups, carbon, bone char and ion exchange resins can be used. In the case of ion exchange, anion exchangers rather than cation exchangers, remove colorant. In your analyses, using anion exchange columns, do you find that color constituents in the syrups interfere with the analyses?

de Bruijn: No, we have not observed any interference from color components - perhaps because of the pH of the mobile phase.

Question: I am a little surprised that in the comparison of results by calcium and magnesium, magnesium gives better results. I would like to know what you plan for further study on this point, if I may ask without requesting confidential information.

de Bruijn: I've nothing particular planned. There is always the difficulty that when one component is determined by different methods, results may be different. This does require further study.

APPLICATION OF A MODIFIED HAZE METHOD FOR "DEXTRAN" TO ASSESS FROST DAMAGED SUGARBEET

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ABSTRACT

A reduction in sugarbeet quality, resulting from frost damaged tissue, is of extreme concern to both growers and sugarbeet processors. Biodeterioration (rot) of sugarbeet tissue, affected by freeze-thaw conditions, has been attributed to the vital activities of microorganisms that feed on sucrose. This type of deterioration is usually associated with a rapid decline in the sucrose content of sugarbeet accompanied by a rapid accumulation of high molecular weight polysaccharides.

Rot resulting from frost damaged sugarbeet is generally associated with sucrose losses which are in turn accompanied by the formation of reducing sugars, colorants and microbiological metabolites such as lactic acid, acetic acid and dextran. Deterioration can therefore be monitored by the loss or formation of these compounds.

Since frost damaged sugarbeet is nearly always characterized to some extent by the formation of dextran, a method, based on a modification of the SPRI Rapid Dextran Screening Test haze determination, was developed for assessing dextran concentration in sugarbeet.

The modified method was shown to provide rapid, semi-quantitative measurements for "dextran" concentrations in aluminum sulfate filtrates of frost damaged sugarbeet. The modified haze method was used to screen sugarbeet loads so as to reduce "dextran" concentrations going in to the sugarbeet factory process.

INTRODUCTION

Frost damage to sugarbeets has always been of extreme concern to both the grower and to the processor. Biodeterioration (rot) of sugarbeet tissue, affected by freeze/thaw conditions, is generally associated with a rapid decline in the sucrose content. This deterioration is usually accompanied by a rapid accumulation of high molecular weight polysaccharides.

Rot resulting from frost damaged sugarbeets is generally associated with sucrose losses which are accompanied by the formation of

reducing sugars, colorants, and microbiological metabolites such as lactic acid, acetic acid, and dextran. Deterioration can be monitored by the formation and/or loss of these compounds.

A rapid method was needed to determine the extent of the deterioration of the sugarbeet crop which was exposed to freezing conditions during late October, 1992, at the Hereford, Texas plant.

DEXTRAN FORMATION

Dextran formation has long been associated with deteriorated sugarcane and identified in sugarbeet tissue subjected to freeze-thaw conditions.(2,4,7,11,12,13,15,17,18,19,20). This type of deterioration is usually associated with a rapid decline in the sucrose content and the accompanying manufacturing problems associated with high molecular weight material; i.e., dextrans.

Dextran is an α-1,6 linked glucose polymer with random α-1,3 and α-1,4 branching. Dextran is produced from sucrose by a large number of microorganisms (8,14). The most prevalent of these is Leuconostoc mesenteroides, which is generally accepted as the principle source of dextran in sugarcane products.

Many researchers have developed methods for the determination of dextran in various cane samples and products (5,6,9,10,13,17,18). Nicholson and Horsley (13) developed a method for the determination of dextran in cane juices and sugar products which was subsequently revised by Hidi et al. (7). The method is commonly known as the alcohol "haze" method, or CSR method (12). Sugar Processing Research Institute, Inc. further improved this method to produce a rapid dextran screening test for sugarcane juice and raw sugar (3).

Described herein is a modified alcohol precipitation, or haze method for the rapid determination of dextrans in sugarbeet aluminum sulfate filtrates.

MATERIALS AND METHODS

Composition of sugarbeet aluminum sulfate filtrate alcohol precipitate.

Aluminum sulfate filtrate (1/2 normal weight) samples of sugarbeets of varying degrees of frost damage were prepared using 26.0 grams of brei and 178.0 mL of 0.3% aluminum sulfate solution. Polysaccharide material was precipitated in 80% ethyl alcohol and the precipitate centrifuged to produce a pellet. The pellet was re-suspended in water and dissolved in acetic acid. The solution was then ultrafiltered using a 10,000 Dalton membrane. In order to

test for the presence of levan, a fructose polymer also associated with frost damaged sugarbeet, the solution was adjusted to 0.1 N by addition of equal volume of 0.2 N oxalic acid and hydrolyzed at 100°C for 30 minutes (19). Under these conditions levan is quantitatively hydrolyzed to fructose, but dextran is not affected (1). Oxalic acid hydrolyzed material was subjected to high performance ion chromatography (HPIC). Results indicated traces of fructose (<0.05% of the original pellet weight) corresponding to a slight presence of levan. Even though earlier researchers suggested that levan did not interfere with the determination of dextran by haze analysis, the sensitivity of their measurement for fructose may not have been adequate for trace levels (1,19).

Oxalic acid treated freeze-dried material was subjected to enzyme treatment with dextranase. Decreased molecular weight as shown by gel permeation chromatography (GPC) and solution viscosity was evident. The oxalic acid treated material was analyzed by HPIC following hydrolysis by trifluoroacetic acid. Results showed greater than 99% glucose and no trace of fructose. These results indicated dextran as the principle polysaccharide in the freeze-dried material originating from the aluminum sulfate filtrates of frost damaged sugarbeet.

Even though trace concentrations of fructose were observed in the original alcohol precipitate (<0.05%), the relative concentrations of the corresponding levan would not produce a significant effect in the final dextran concentration. Furthermore, as levans are also associated with microbial infection of frost damaged sugar-beets, levans plus dextrans give a truer indication of deteriorated root (2,11,15,19,20).

Influence of molecular weight

Five commercial dextrans of molecular weights 40K, 70K, 242K, and 500K (Sigma Chemical Co.) were evaluated as a measure of the effect of molecular weight on alcohol haze formation rate. Five different concentrations of each dextran type were prepared in 0.3% aluminum sulfate for development of standard curves.

Haze formation was initiated by precipitating dextran at approximately 80% ethyl alcohol concentration by using 0.5 mL of a standard and 2.5 mL of 95% ethyl alcohol. The haze was measured following a two minute precipitation reaction at room temperature and agitation in a one centimeter spectrophotometer cell read at 720 nm. Absorbance was plotted against dextran concentration to develop standard curves. Dextran from aluminum sulfate filtrates of frost damaged sugarbeet brei were compared to these standard curves to determine the most effective dextran molecular weight to use as a standard.

Determination of dextran in aluminum sulfate filtrates

1. Pipette 0.5 mL of a 1/2 normal weight aluminum sulfate brei filtrate, produced using 0.3% aluminum sulfate, into a clean one centimeter spectrophotometer cuvette.

2. Pipette 2.5 mL of 95% ethyl alcohol into the same cuvette and mix by inverting the cuvette covered, with a parafilm seal, 3 times.

3. Start timer for two minutes and place the cuvette containing the mixed solution into a cuvette holder.

4. After two minutes mix by inverting cuvette and read absorbance at 720 nm.

Preparation of standard dextran curve

1. Prepare a stock solution of 242,000 M. W. dextran containing 25 mg/mL of the dextran in distilled water.

2. Pipette, into four different 100 mL volumetric flasks 2, 4, 6, 8 mL of the dextran stock standard.

3. Bring each of the 100 mL volumetric flasks to volume and mix.

4. The flasks represent 500, 1000, 1500, 2000 mg/L of dextran for the 2, 4, 6, 8 mL additions respectively.

5. 0.3% aluminum sulfate solution and 2.5 mL of 95% ethyl alcohol is used as a blank for the zero set point on the spectrophotometer at 720 nm.

6. The standard curve is prepared by repeating steps 1 through 4 under "Determination of dextran in aluminum sulfate filtrates".

7. A linear regression equation is developed for absorbance against mg/L dextran. This equation is used to calculate mg/L dextran in the aluminum sulfate filtrate samples.

RESULTS AND DISCUSSION

Figure 1 shows the comparative curves for standard 242,000 M.W. dextran and dextran from frost damaged sugarbeet aluminum sulfate filtrates.   Figure 2 shows curves for standard dextrans of different molecular weights and frost damaged sugarbeet aluminum sulfate filtrates.   These comparative curves illustrate the reason

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for the choice of 242,000 M.W. dextran for the development of standard curves for routine analysis.

Table 1 shows the reproducibility of the modified haze method using ten replications of a moderately deteriorated sugarbeet sample. Dextran concentrations were determined using the 242,000 M.W. standard curve.

A linear regression analysis of the 242,000 M.W. dextran standard curve is shown in Table 2. Excellent correlation is indicated at R = 0.99.

Table 3 gives recoveries of 242,000 M.W. dextran added to an aluminum sulfate filtrate of a defined dextran concentration. The dextran concentration in the aluminum sulfate filtrate sample is the mean of the reproduciblity trial. As illustrated, recoveries are good, at greater than 98% for all trials.

The method was shown to be highly reproducible and relatively specific for dextran in aluminum sulfate clarified sugarbeet filtrates.

FACTORY EXPERIENCE AND APPLICATION OF DEXTRAN TEST

The crop was approximately 30% harvested when it was exposed to freezing conditions. To further add to the deteriorating condi- tions, wet and warm weather followed the freezing weather.

The wet conditions led to stockpiling six to seven days' supply of sugarbeets in an attempt to outlast the wet weather. As the factory processed the stockpile it was evident that storing sugarbeets which had been exposed to the extreme conditions of late October would not be feasible. The factory experienced extreme settling and filtration problems with beets from this pile.

Figure 3 shows the slice rate, production rate and the on-hand inventory by day for the month of November. As the weather warmed up and the on-hand beet supply exceeded 30,000 to 35,000 tons, or three to four days' slice, the daily sugar production started declining. On November 16 harvest was suspended because of the large inventory of beets on hand and the problems associated with processing deteriorated beets.

The storage piles were also showing the effects of deteriorating sugarbeets. Streams of juice were running from under the storage piles. The storage piles were showing signs of shrinking in height.

The next nine to ten days were an operating nightmare for the perations department. Second carbonation filtration cycles were as short as five minutes for days on end. With color and turbidity

problems, the daily sugar production dropped to less than 50,000 lbs. The slice rate was somewhat better due to availability of thick juice storage.

Harvest resumed on November 26 with the intent keeping only enough beets in storage to allow a few days slicing. The sugar production increased to an acceptable level over the next few days. Filtration problems were the greatest when the beets were stored longer than 36 hours.

The beets were becoming more deteriorated as harvest continued. The slice rate along with the production rate also declined as harvest continued (see Figure 4).

On December 17 the tare lab began to use the modified "haze" test to determine dextran content in the sugarbeet extract (aluminum sulfate filtrate) along with the standard analyses run in the tare lab.   Over 200 samples were analyzed for dextran with results varying from a few hundred ppm to a few thousand ppm; however, the majority were less than 700 - 800 ppm (see Table 4).

Using a nominal grouping technique to identify that 20% of the causes are resulting in 80% of the problems, Figure 5 shows that beets containing 800 ppm dextran and greater would be in the 20%, causing 80% of the problems.

On December 22 harvest was again delayed due to rain (see Figure 6). The remaining beets on hand were sliced and thick juice that had been stored from earlier in the campaign was processed.   The juice that was processed from the beets that were in storage for seven days didn't process any better the second time.

With 800 ppm dextran established as the cut off point, the actual growers' samples were analyzed for dextran.   Any contract at the end of the harvest day that exceeded an average of 800 ppm was not allowed to deliver any more beets.   Table 5 is a typical sample of the results of beets harvested after January 3, 1992. The results include the average dextran, contract number, and the individual dextran analysis of each sample run.

Harvest continued through January 16.   During this period the number of contracts that were eliminated ranged from 2% to 23% daily.

As harvest progressed, using the 800 ppm cut off point, the number of contracts eliminated increased each day.   However, when approximately 20% of the contracts delivered was eliminated, the next day or two less contracts would be eliminated.   Figure 5 indicates the levels of dextran in the remaining crop to be harvested was reduced.

After a few days,the slice and production rate started declining until finally there was not an adequate supply of beets to maintain operations on a steady basis and the campaign was ended. See Figure 6.

The use of the modified "haze" method allowed beets which could be processed to be harvested for a longer period of time. These beets allowed an additional 130,000 cwt of sugar to be extracted that would have otherwise been left in the fields.

CONCLUSION

The modification of the "haze" dextran test proved to be a tool that could be utilized in time of adverse conditions where dextran is a major processing problem. The "haze" dextran testing also allowed the grower and the processor to select beet that could be harvested and processed rather than leave them in the field, so that both could benefit.

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Table 1.   Reproducibility trials

Trial No.	Abs. (720 nm)	Dextran (mg/L)
1	0.095	908
2	0.097	927
3	0.095	908
4	0.095	908
5	0.097	927
6	0.096	918
7	0.098	937
8	0.096	918
9	0.097	927
10	0.095	908
Mean	0.096	919
S.D.	0.001	11

Table 2.   Linear regression of 242,000 M.W. dextran

Regression Output:

Constant	1.966554
Std Err of Y Est	6.968277
r^2(R)	0.999942
No. of Observations	5
Degrees of Freedom	3
X Coefficient(s)	9541.429
Std Err of Coef.	42.0515

Table 3.   Recovery of standard T-242 dextran added to
brei filtrates.

Trial No.	1	2	3	4	5
Dextran mg/L	919	919	919	919	919
mg/L added	100	200	300	400	500
Tot. mg/L observed	1003	1109	1211	1309	1415
% recovery	98.4	99.1	99.3	99.2	99.7

Table 4.

SAMPLE NUMBER	PPM-DEXT	SAMPLE NUMBER	PPM-DEXT	SAMPLE NUMBER	PPM-DEXT
1	250	71	1708	141	563
2	231	72	1613	142	676
3	572	73	440	143	714
4	421	74	440	144	515
5	402	75	477	145	298
6	402	76	619	146	1131
7	600	77	392	147	563
8	591	78	506	148	1036
9	563	79	430	149	307
10	402	80	477	150	364
11	345	81	373	151	724
12	231	82	487	152	307
13	629	83	1292	153	402
14	231	84	705	154	515
15	496	85	373	155	335
16	2134	86	1131	156	600
17	477	87	421	157	487
18	250	88	553	158	307
19	856	89	440	159	421
20	1282	90	496	160	638
21	269	91	525	161	941
22	657	92	288	162	733
23	279	93	515	163	430
24	458	94	1027	164	402
25	695	95	241	165	742
26	563	96	525	166	506
27	458	97	440	167	695
28	364	98	383	168	411
29	383	99	487	169	458
30	572	100	364	170	733
31	279	101	1415	171	657
32	458	102	411	172	648
33	714	103	288	173	335
34	383	104	1509	174	506
35	582	105	335	175	449
36	326	106	572	176	619
37	392	107	600	177	1027
38	449	108	525	178	809
39	582	109	1803	179	629
40	440	110	487	180	733
41	733	111	231	181	761
42	430	112	477	182	705
43	402	113	619	183	1121
44	477	114	610	184	392
45	951	115	468	185	563
46	373	116	515	186	619
47	525	117	515	187	411
48	307	118	648	188	714
49	506	119	894	189	1131
50	392	120	421	190	1329
51	345	121	818	191	676
52	241	122	1131	192	468
53	411	123	572	193	1102
54	619	124	837	194	449
55	515	125	307	195	411
56	298	126	373	196	600
57	572	127	298	197	534
58	288	128	487	198	440
59	477	129	430	199	421
60	506	130	430	200	879
61	1356	131	383		
62	383	132	383		
63	1727	133	534		
64	1689	134	496		
65	364	135	288		
66	761	136	477		
67	582	137	525		
68	468	138	468		
69	402	139	563		
70	354	140	364		

Table 5. Dextran daily results January 10, 1992

AVERAGES	CONTRACT NO.	HAZE ABS.	PPM DEXTRANS
560	4	0.079	828
	4	0.056	611
	4	0.054	592
	4	0.048	535
	4	0.034	403
	4	0.033	393
393	21	0.041	469
	21	0.025	318
390	81	0.037	431
	81	0.034	403
	81	0.027	337
400	93	0.037	431
	93	0.036	422
	93	0.035	412
	93	0.027	337
330	108	0.033	393
	108	0.029	356
	108	0.022	289
	108	0.021	280
365	112	0.037	431
	112	0.023	299
374	147	0.038	441
	147	0.024	308
576	152	0.069	734
	152	0.046	516
	152	0.042	478
313	160	0.026	327
	160	0.023	299
478	178	0.042	478
1334	185	0.168	1669
	185	0.097	998
682	224	0.092	951
	224	0.035	412
743	241	0.070	743
407	274	0.051	563
	274	0.042	478
	274	0.039	450
	274	0.029	356
	274	0.025	318
	274	0.021	280
1130	315	0.149	1490
	315	0.073	771
1045	342	0.102	1045
897	433	0.180	1782
	433	0.078	819
	433	0.046	516
	433	0.041	469
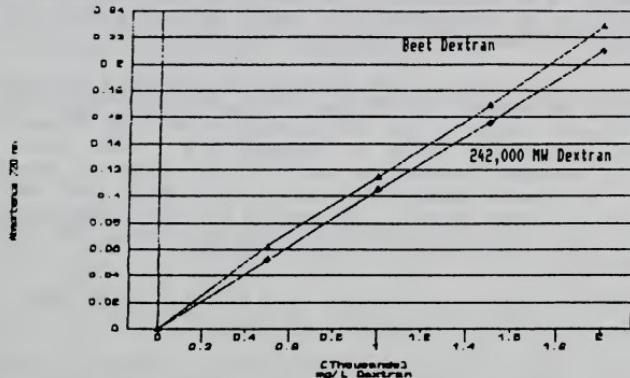
Figure 1. Dextran Comparative Curves
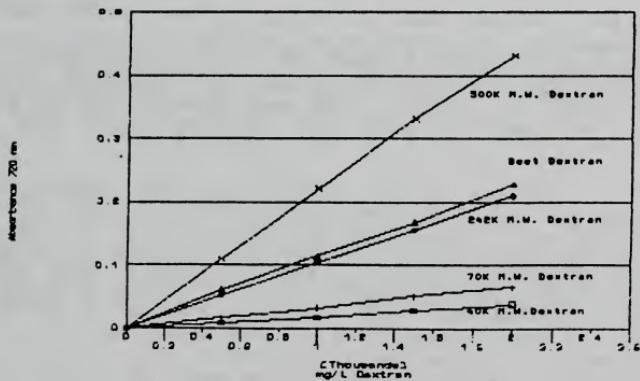
Figure 2. Dextran Standard Curves

DISCUSSION

Question: A comment: would you outline Holly's initial efforts, before this test, to correlate dextran levels with lactic acid, pH, conductivity or invert concentrations, when we were trying to find a rapid estimate for dextran content.

Duncan: At first, we tried a YSI analyzer to measure glucose and lactic acid; we measured color and conductivity; we tried to correlate a visual test. The correlations on all these with dextran levels were very poor - perhaps 0.2. The only good correlation was between processing ability and beets as harvested was dextran. A visual test was very inaccurate, and was not without subjective human error. We worked out the test described here, and talks were held between grower and processor how the test should be applied.

Question: I don't really understand how this test is applied in the tare house. How long does this test take?

Duncan: It takes about 2 minutes. The aluminum filtrate sample is mixed with alcohol and allowed to stand for two minutes. Then turbidity is read, at 720 nm. The ppm dextran was determined from this turbidity reading.

Question: Did you keep the load of beets waiting for 2 minutes?

Duncan: No; the load had been delivered at that point, and was then the processor's. A grower might deliver 5 loads that day. The average dextran in those 5 loads would determine whether that grower could dig for the next day or not. We accepted the five loads on the first day; but, if the same grower's loads showed dextran above 800 ppm, no more beet were accepted from that contract.

Question: To jump the gun on one of the SPRI papers to be presented tomorrow: we've analyzed the dextran from frozen beets that goes into the white sugar, and have identified it as a product of Leuconostoc mesenteroides B-512F strain, the same organism and the same dextran as found in sugarcane factories. This finding means we have a familiar problem to deal with -not a whole new phenomenon.

SUCROSE INVERSION PRODUCTS: ANALYTICAL INCONSISTENCIES DUE TO DI-D-FRUCTOSE DIANHYDRIDE FORMATION

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ABSTRACT

Products from sucrose hydrolysis in the presence of a hetero-genous
catalyst (cation exchange resin in the acid form) were found to
contain apparent unequal quantities of fructose and glucose.
Investigation of product composition by gas chromatography revealed
several by-products which were identified as di-D-fructose
dianhydrides, previously reported from fructose dehydration in the
presence of acid. Fructose/glucose solutions (invert syrups)
produced by such hydrolysis may contain small amounts of these
anhydrides depending on the hydrolysis conditions. The observed
products may interfere with hydrolysis monitoring by liquid
chromatography or polarimetry.

INTRODUCTION

Heterogeneous catalysis of sucrose hydrolysis using acid form
strong cation exchange resins can be used to produce fructose-
glucose solutions (invert syrups) (1,2). Such processes have the
advantage of continuous operation without the acid catalyst's
appearing in the product. During a series of such tests in our
laboratory anomalous analytical results were obtained for hydroly-
sis products. Sample analysis by high performance liquid chroma-
tography (HPLC) generally gave fructose-glucose ratios significant-
ly lower than one. For example, although sucrose inversion
(hydrolysis) should give equal levels of fructose and glucose, a
typical sample was indicated by HPLC to contain (on a total
carbohydrate basis): 47.8% fructose, 50.3% glucose, and 1.9% of a
disaccharide assumed to be sucrose. The optical rotation of such
samples did not agree with the HPLC analysis but was typically more
levorotatory than predicted from specific rotations. Further
investigation of these anomalies led to some interesting observa-
tions on fructose reaction products and their effect on analytical
results.

METHODS AND MATERIALS

Sucrose inversion experiments were carried out by passing sucrose solutions (57% w/w) at 38-40°C through a column of strong acid cation exchange resin (Bayer OC-1052) in the acid form at 0.4 bed volumes per hour.

HPLC analyses were performed using water at 80°C as eluent, a Bio-Rad HPX-87C column, and a Waters chromatograph equipped with R401 differential refractometer.

Gas chromatography (GC) analyses were carried out with a Hewlett-Packard 5890A instrument equipped with a flame ionization detector and a 10 m HP-3 column (0.53 mm inner diameter). Carbohydrate samples were silylated with trimethylsilylimidazole prior to injection. Salicin (2-hydroxymethylphenyl B-D-glucopyranoside) was used as the internal standard.

Optical rotation measurements were made with a Sucromat automatic saccharimeter in a jacketed cell thermostatted to 20°C ±0.1°C.

Carbohydrate analysis by ion chromatography was performed using a Dionex system with a pulsed amperometric detector and an HPIC AS6-10μ column. Samples were eluted with 0.07 N sodium hydroxide at 1.3 ml/min.

RESULTS AND DISCUSSION

Since fructose to glucose ratios in samples were less than the expected value of one, it was assumed that fructose was being formed by sucrose hydrolysis and subsequently destroyed in some further reaction(s). Fructose is known to form 5-hydroxy-methyl-furfural (HMF) under acidic conditions and, in fact, HMF has been reported in products of heterogeneous sucrose inversion [1,2]. In our tests, inverted samples with differences between fructose and glucose levels of up to 2 g/100 g carbohydrate were found, however, to have an ultraviolet absorbance at 283 nm consistent with only 200-300 ppm of HMF. This HMF level would account for only approximately 0.04 g of fructose loss.

Although numerous inversion product samples obtained had shown low fructose-to-glucose ratios the most extreme effects were noticed in samples with long exposure to acid form ion exchange resin. A single sample that had been in contact with resin during an eight hour shutdown of the inversion column was selected for further tests because of the high difference between glucose and fructose levels. This sample was indicated by HPLC to consist of (in g/100 g carbohydrate): fructose, 43.8; glucose, 54.1; oligosaccharides, 2.1. Optical rotation of the solution (200 mm cell, 20.0°C, concentration = 60.4 g solids/100 g solution) was found to be

-30.5°. Calculating specific rotation from the HPLC levels and published specific rotations, assuming the oligosaccharide peak is residual sucrose, gave a value of only -17.4°. The high measured optical rotation of the inverted material lead to the conclusion that the product of fructose destruction cannot be optically inactive or dextrorotatory but must be another levorotatory substance.

Fructose forms a series of dimeric dianhydrides, known as diheterolevulosans, upon treatment with acid (3). Configurations shown in structures (1-4) were established for diheterolevulosans I-IV by Binkley (4,5). A sample of crude mixed di-D-fructose dianhydrides was prepared using the method described by Hilton (3) carried only as far as the neutralization of acid over an anion exchange resin. This material was used for chromatographic comparison with inverted sucrose syrups.

The invert sample under investigation gave the gas chromatography plot shown in Figure 1 using temperature programming conditions of 165°C for 15 minutes followed by a 15 degree per minute increase to 235°C. Peaks at 5-11 minutes are due to various glucose and fructose anomers; the 17.49 minute peak is the internal standard, and peaks in the 16-24 minute time span are probably disaccharides. Under identical conditions sucrose gives a peak at 19.34 minutes and the peak at 19.58 minutes in Figure 1 was assumed to be a slightly displaced peak due to residual sucrose. The dianhydride mixture shows, however, in addition to residual fructose at 4-7 minutes, two major peaks at 18.93 and 19.55 minutes (Figure 2) which are at retention times essentially identical to the two major disaccharide peaks in Figure 1. Thus the 19.58 minute peak in inverted material is probably not sucrose but a di-D-fructose dianhydride. To establish definitely that sucrose and the material giving the 19.58 minute peak could be distinguished under the GC conditions, sucrose was added to a sample of the dianhydride mixture. The resulting chromatogram (Figure 3) shows sucrose falling between the two dianhydride peaks to give a broad peak with a small shoulder. A chromatogram of the crude diheterolevulosan mixture under the same GC conditions is shown in Figure 4. Evidently the inverted sucrose sample contains no sucrose detectable by GC but does contain the principal two components of the diheterolevulosan mixture.

Further purification of the crude diheterolevulosan mixture by crystallization from methanol according to the method of Hilton (3) gave a crystalline solid stated by Hilton to be an approximately 2:1 mixture of diheterolevulosan II (which has fructofuranose structure 2 according to Binkley (4) and the di-D-fructopyranose isomers [diheterolevulosan I (α,α structure $\underline{1}$ according to Binkley) and diheterolevulosan IV (β,β structure $\underline{4}$ according to Binkley)]. This material produced the same two GC peaks seen at 18.93 and 19.55 minutes in the crude mixture and in the invert sample. The

presence of only two peaks suggests that either the closely related dianhydrides (1) and (4) are not separable under the GC conditions or only one is present. In any event the inverted sucrose solution seems to contain dianhydride (2) and one or both of the isomeric compounds (1) and (4). The small peaks eluting between 16 and 24 minutes in the invert GC plot are possibly other disaccharides formed by dehydration since both fructose and glucose at high concentrations are known to produce a variety of such substances in the presence of acid (6).

The identity of the substances in the invert sample with dianhydride components was confirmed using a second chromatographic system. Ion chromatography with 0.07 N sodium hydroxide eluent on an anion exchange column using pulsed amperometric detection gave two major peaks, 3.09 and 4.07 minutes, for the crystalline dianhydride mixture. An invert sample showed, in addition to glucose and fructose at 4.97 and 5.49 minutes respectively, two small peaks at 3.10 and 4.09 minutes, identical to the dianhydride retention times. Interestingly the dianhydrides elute before fructose and glucose while disaccharides with a single glycoside linkage (such as sucrose) elute after monosaccharides.

Our initial unexpected HPLC results were explained by analysis of the crude di-D-fructose dianhydride mixture under the conditions used for invert samples. Four significant peaks (Figure 5) were obtained: 10.1 minutes (2% of total peak area) at the usual disaccharide retention time; 11.8 minutes (34.8% of area); 13.5 minutes (22.2% of area); and a fructose peak at 15.8 minutes (39.9% of area). The 11.8 minute and 13.5 minute peaks are evidently the two major dianhydride components which are retained more strongly than most disaccharides. The 10.1 minute peak is most likely due to other disaccharide reaction products. The two dianhydride peaks bracket the normal glucose retention time of 12.3 minutes. Addition of a high concentration of glucose to the dianhydride mixture gave a chromatogram with the 11.8 minute peak totally obscured and the 13.5 minute peak as a small shoulder on glucose (Figure 6). Any dianhydrides present at low levels in an invert sample would likely be included in the area of the glucose peak (see chromatogram of invert sample, Figure 7).

Based on the GC and HPLC data it can be concluded that the invert sample examined contains:

1)   No detectable sucrose (GC).

2)   43.8 g fructose/100 g carbohydrate (HPLC).

3)   2.1 g unknown oligosaccharides/100 g carbohydrate (HPLC, assuming detector response is similar to other carbohydrate components).

4) Di-D-fructose dianhydrides at a level of approximately 4 g/100 g carbohydrate, assuming the original glucose analysis of 54.1% is due to the theoretical glucose level plus dianhydrides.

5) 50 g glucose/100 g carbohydrate.

Note that the assumption that only fructose forms dehydration products explains the fact that the 2.0% oligosaccharide content plus the 4.1% difference between the actual and theoretical HPLC glucose peak areas agrees well with the 6.2% difference between theoretical and actual fructose peak areas (neglecting the relatively small molecular weight changes due to dehydration).

Since the concentrations of specific dianhydride components and identity of other oligosaccharides present in the invert sample are unknown, a predicted optical rotation cannot be calculated for comparison with the measured value. However, dianhydrides (1-4) are known to be levorotatory. Thus the production of levorotatory products from fructose plus the falsely high glucose concentration obtained by HPLC of the invert sample explains the lack of agreement between the rather high negative optical rotation measured and the less negative value predicted from the initial HPLC results.

CONCLUSIONS

During the production of invert syrups by heterogeneous catalysis on acid form cation exchange resins several by-products are formed in detectable amounts, particularly at long reaction times. By-products include several members of the series of dimeric fructose dianhydrides known as diheterolevulosans along with smaller amounts of several compounds thought to be disaccharides.

The presence of di-D-fructose dianhydrides if not taken into account can influence analytical results obtained by either HPLC (on calcium form cation exchange columns) or polarimetry. On HPLC the substances co-elute with glucose producing erroneously high glucose peak areas. The dianhydrides are levorotatory and thus may introduce errors into any attempt to quantitate inversion using polarimetry.

ACKNOWLEDGEMENTS

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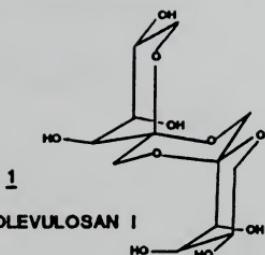
1
DIHETEROLEVULOSAN I
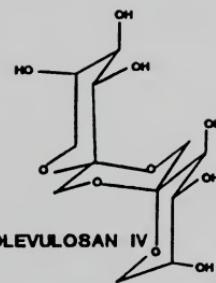
4
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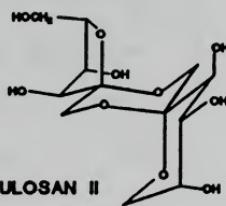
2
DIHETEROLEVULOSAN II
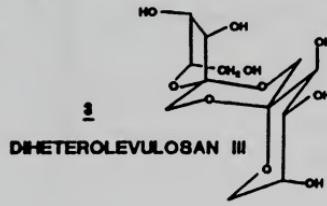
3
DIHETEROLEVULOSAN III

SPRI
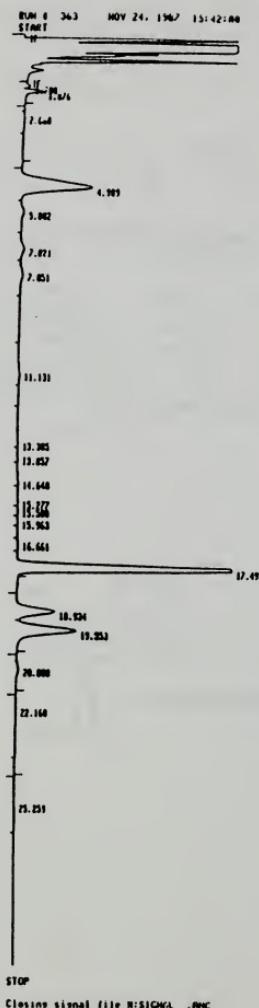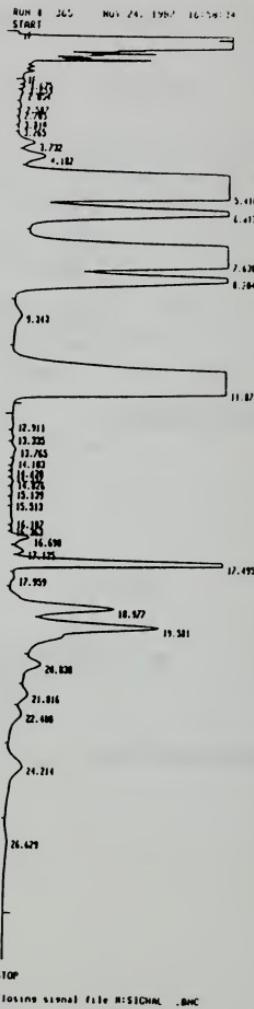
FIGURE 1
Invert Sample

FIGURE 2
Crude Diheterolevulosan Mixture

104
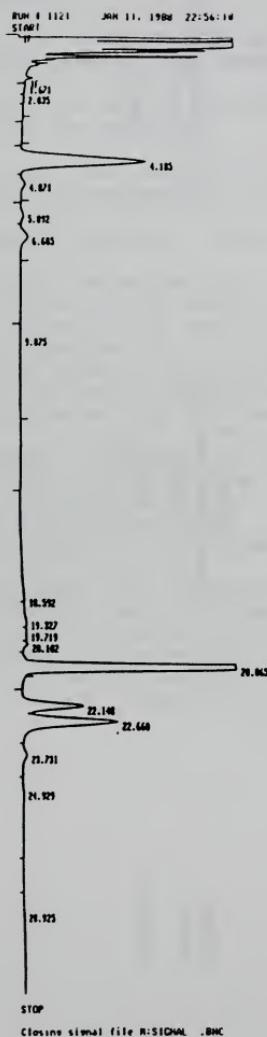
FIGURE 4

Diheterolevulosan Mixture
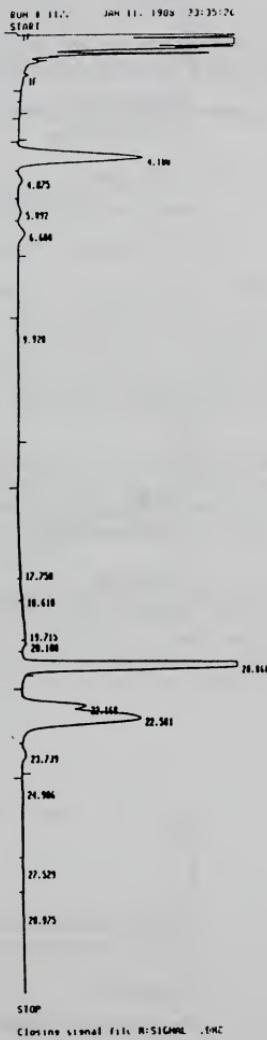
FIGURE 3

Diheterolevulosan Mixture with Added Sucrose
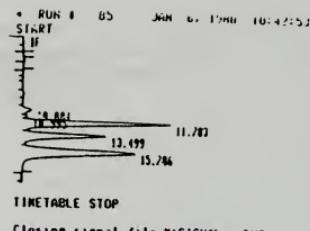
FIGURE 5

Crude
Diheterolevulosans
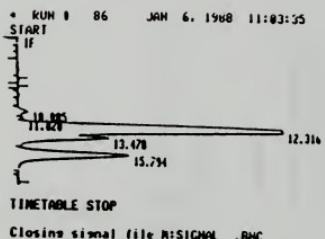
FIGURE 6

Crude
Diheterolevulosans
+ Glucose
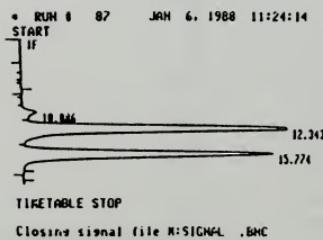
FIGURE 7

Invert
Sample

DISCUSSION

Question: Congratulations on the closeness of your mass balance. Have you looked at the formation of kestoses during the hydrolysis?

Rearick: We did this work 4 years ago and, to my memory, we did not look for kestoses.

Question: They might account for the missing 2% - they are known to form from enzymatic or acidic hydrolysis .

Rearick: We did not search for kestoses exhaustively.

Question: We agree that the kestoses, and perhaps other oligosaccharides are probably formed in small quantities; but may not show up on IC that uses only sodium hydroxide as eluent, with no sodium acetate added to speed up their elution times. Thank you for this work on difructose dianhydrides - you've identified some peaks that were heretofore unknown to us, peaks that come out before glucose and fructose.

Question: A comment: in recent literature it has been noted that only two of the difructose dianhydrides form in concentrated acid with fructose, and that only one of those is stable in dilute aqueous acid solutions. This may be the reason for your unusual HPLC profile for difructose dianhydrides.

In addition, the hydrolysis of sucrose forms a fructose carbocation that, in dilute solutions, will react with water to form fructose; in concentrated solutions, it will seek any hydroxyl group and so eventually form the kestoses. It could also possibly form a reducing disaccharide. You have several GC peaks unidentified - all of these are possibilities.

ENVIRONMENTAL ASPECTS OF CARBONATATION MUD DISPOSAL *p*

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ABSTRACT

The nature of the problem in the United Kingdom situation is
discussed, and data on the chemical changes which occur during
storage of carbonatation mud are presented. Some laboratory
studies on factors which control odour production are described,
and the options for reducing odour on site are discussed.

INTRODUCTION

British Sugar's system for the disposal of factory carbonatation
mud (lime) follows the long-established procedure of lagooning
during the processing season followed by a drying-out period in
the lagoon. The partially-dried lime is then excavated during July
and August and sold to farmers for land application to correct acid
soil conditions and provide soil nutrients. When sold, the lime is
typically about 50% dry matter.

This system may lead to the evolution of objectionable odours from
the lime ponds during the processing season, extending into the
off-season until a crust forms which seals in odour. A second
release of odour may follow when the lime is excavated.

Experience shows that carbonatation mud odours vary from year to
year and also vary between factories. Off-site odours may range
from negligible to substantial, and can elicit complaints from
local populations. Local Authority pressure, ever increasing as the
public becomes more aware of environmental issues and demands
cleaner air conditions, may then require that steps are taken to
prevent odour release.

In the UK, odour emission is controlled under the Environmental
Protection Act, 1990, Statutory Nuisance and Clean Air Order, under
which Local Authorities may serve, or an aggrieved person or
persons may apply to the Courts for, an Abatement Order against the
offender, which will require abatement or prevention of the
nuisance and may specify the means of compliance with the order. As
there are no specified odour concentration limits in operation in
the U.K., companies may be required to make large investments to
prevent emission on the basis of judgement of nuisance and not
necessarily on factual information.

British Sugar's policy on improvement of environmental impact at factories has focussed attention on the cause and control of factory odours. Significant progress has been made as a result of studies of odours from water treatment systems and animal feed driers, but, before now, carbonatation mud odour, though widely recognised, has not been extensively studied within British Sugar.

Lime odour at factories may be avoided by pressing to high dry substance and quitting the material from the site during campaign, a practice used at many European factories. Trials at a UK factory have shown that lime pressing is not necessarily sufficient to prevent biodegradation and odour formation in the product. The odour problem may be transferred to the farm or, where U.K. agricultural practice necessitates storage of lime at factories, may remain unsolved at the factory.

As lime odours are known to be variable and some sites are free from problems, it was considered that research into factors which affect the biodegradation and odour formation of carbonatation mud might provide insight into the control of odour and lead to stable lime or lower-cost alternatives to lime pressing. This paper reports progress in studies of the anaerobic degradation of stored carbonatation mud, undertaken to ascertain the causes of odours and to identify management options for control of lime odour.

ANAEROBIC DEGRADATION.

Lime contains, in addition to precipitated calcium carbonate, residual sugar, insoluble calcium salts, precipitated proteins and polysaccharides, with some beet particulate material which is not removed by raw juice screening. The typical organic content is approximately 21% on dry substance and, unless steps can be taken to sterilize the lime completely during storage and dewatering, anaerobic biodegradation of this organic material will occur.

Anaerobic breakdown of organic materials is achieved by complex pathways involving the participation of several groups of micro-organisms operating under optimum conditions (1). These groups of organisms are affected by environmental conditions and many intermediates and end-products can result.

Despite the complexity of the inter-related biochemical reactions, the general pathway of the complete degradation of organic material to methane and carbon dioxide has been established (1,3). It follows principal stages which are outlined in Figure 1. These stages are:

1. hydrolysis of complex organic materials such as polys-accharides and proteins to simple organic compounds such as

sugars, amino-acids and alcohols. This hydrolytic stage is normally relatively slow.

2. an acidogenic stage, in which the hydrolysis products are converted into lower aliphatic acids, including acetic acid, carbon dioxide and hydrogen. This stage proceeds rapidly under favourable conditions. Sucrose is directly assimilable by acidogenic bacteria and therefore, at the sucrose concentrations present in lime (up to around 15,000ppm), high concentrations of acids can build up rapidly and may exert inhibitory effects on methanogenic bacteria which control the third stage.

3. the final stage, methanogenesis, converts acetic acid into methane and carbon dioxide, i.e. odourless products. Methanogenic bacteria are generally the most sensitive to the effects of environmental stress and their activities are much slower than those of other organisms present.

If conditions are unfavourable for this pathway to proceed smoothly through to methane, there may be a build-up of such intermediates as acetic acid if methanogenic activity is suppressed, or odorous longer-chain fatty acids if acetogenic bacteria are inhibited. In addition, competing reactions can occur which lead to other end-products. For example, if sulphate is not a limiting factor, sulphate-reducing bacteria may compete with methanogens for hydrogen produced by acetogenesis to give hydrogen sulphide rather than the normal odourless end-product methane (4).

This general background suggests that the main influences on odour formation during storage of carbonatation mud in lagoons are likely to be concentrations of key nutrients and conditions which regulate the speed and extent of activity of certain micro-organisms.

GENERAL CHARACTERISTICS OF LIME BIODEGRADATION

Factory Studies 1990/91

A detailed analytical study was undertaken over the complete lime storage cycle at two factories to identify and measure selected odour compounds and, if possible, to establish the cause of the odour formation. One factory (identified as A in the results) had a recent history of lime odour problems; the other (identified as B) was studied (to a lesser extent) to provide comparative data from a site with no recent lime odour problems.

Whilst the ponds were being filled, sampling was restricted to the surface layer (0.3 metres depth maximum) as a practicable method for deeper sampling was not available. When the mud had dried sufficiently to allow access to the complete pond surface, samples

were drawn from depths up to 5 metres, using a hollow probe auger. Over the campaign period, samples were taken at 14 day intervals, then at monthly intervals until the following July.

The following analyses were undertaken:

    pH
    chemical oxygen demand (C.O.D.)
    volatile aliphatic acids (VFA), by gas liquid chromatography
      (G.L.C.)
    volatile amines, by G.L.C.
    sulphide, by trapping in cadmium hydroxide followed by
      spectrophotometric determination using N-N dimethyl-p-
      phenylenediamine.
    thiol, by trapping in mercuric acetate followed by
      spectrophotometric determination using N-N dimethylamine-
      p-phenylenediamine.
    indoles, by solvent extraction and G.L.C. using nitrogen-
      selective detection.

Results are quoted on sample.

RESULTS

Results of biodegradation in factory lime ponds.

The data are summarised in Table 1, which records mean values of surface layer samples representing four periods from the start of operations (October 90) through to July 91 and mean values of samples representing lime bed depths ranging from 0.5 to 4 metres taken over the period February to July 91. Feed to the ponds was stopped at 18 weeks from the start of the campaign.

The principal changes in the lime were in pH and concentrations of volatile aliphatic acids and key odour compounds sulphide, thiols, indoles and methylamines.

Odour intensity was not measured but a subjective assessment was made, both on-site whilst sampling and of the samples. On-site odours were detectable in the immediate vicinity of the lime ponds during the campaign whilst the lime was wet, but high intensities likely to invoke complaints did not develop at either site during the year 90/91. The odours most consistently present were of ammonia, sulphide and indole, giving a combined odour similar to sewage or manure, albeit only at low to moderate intensities. The odours of thiols and amines were detected intermittently.

Whilst the lime pH was high, the odour at each site was characte-rised as beety, sweet and ammoniacal. When the lime had fallen to below about pH 9, foul odours became apparent, characteristically

of sulphide, indoles, thiols and methylamines. Although high concentrations of volatile fatty acids including butyric were known to be present, their characteristic odours were not observed in the the wet lime, presumably because of stabilisation by salt formation as the pH did not fall appreciably below neutral. If the carbonatation mud was allowed to dry, butyric acid odours became more noticeable.

The general picture for the surface layer is that the lime fell to around pH 8, with the rate of decrease accelerating in the final weeks of the campaign (15-18) and especially once fresh lime addition had ceased. The drop was accompanied, and presumably caused, by VFA production, principally acetic acid, with no higher homologues than butyric acid. The concentrations of sulphide, indole and thiols remained steady and low, but enough to impart readily detectable malodours in samples and on-site. Of amines, only methylamines, mainly trimethylamine, were detected, with steady build-up over the full 35 week period; despite the concentration achieved, the characteristic fishy odours were detected only intermittently on site. The C.O.D. content remained high throughout.

The main differences in the behaviour of the carbonatation mud in the two lagoons were within the beds, rather than in the surface layers. At Factory A, the lime was below pH 8, with VFA content at times exceeding 20g/kg. The lime was black, and samples had pronounced foul odours. At Factory B, on the other hand, the lime remained pale grey, with an odour similar to that of fresh lime cake. It was between pH 8 and pH 11, with mean pH 9.8, and the VFA content did not exceed 6g/kg. Concentrations of malodorants like sulphide and indole remained very low although concentrations of total methylamines were several-fold greater than those at Factory A.

In view of these differences, sampling was extended to the other ten factories. At two factories, lime was approximately pH 9.5 and the odour was relatively fresh, comparable with results found at Factory B. The lime at the other factories was all between pH 7.4 and pH 8.8 and had varying degrees of foul odours similar to Factory A.

Discussion of biodegration in factory lime ponds.

Biodegradation in factory lime ponds is characterised by the maintenance of high levels of C.O.D. over long periods and by the generation of high concentrations of volatile aliphatic acids, accompanied by a fall in pH almost to neutral. In such cases, foul odours are detectable, whereas a fresh lime odour is retained when there is relatively little biodegradation to VFA, so that the lime remains above about pH 9.

These factors indicate that odour occurs in lime ponds because anaerobic degradation is not proceeding to completion via the normal pathway which results in the conversion of C.O.D. to methane and carbon dioxide. It would seem that the pathway is interrupted after the acidogenic stage, resulting in high concentrations of volatile aliphatic acids. Despite this, the presence of calcium carbonate prevents the lime falling below pH 7 so that characteristic volatile aliphatic acid odours are not released. These conditions in the lime lead to the generation of odorous compounds such as sulphides, indoles, amines and thiols. The lack of any significant reduction in C.O.D. supports the contention that the pathway is blocked beyond acidogenesis.

Whilst this undesirable degradation pattern was evident to a varying extent in the majority of the 12 factories, there is evidence that a more stable lime, in which pH remains relatively high and foul odour is not generated, can be obtained even after long storage periods. The results suggest that such conditions correspond to low or virtually no anaerobic activity.

FACTORS AFFECTING BIODEGRADATION

Hypotheses and Initial Experiments.

A research programme was set up to identify major factors which affect biodegradation and to assess the effects of these factors in real lime ponds. From general knowledge of anaerobic processes and observations made above, the following factors were considered most likely to influence the course of biodegradation and hence the end-products:

- organic content of the lime, in particular, the sugar
  content.
- pH
- temperature
- nutrient availability
- anaerobic reaction inhibitors, namely oxygen, added
  VFA, ammonia, sulphate
- bacterial control agents: biocide; hydrated lime
- dry substance.

These factors were investigated initially in a series of laboratory experiments in which carbonatation mud was allowed to undergo anaerobic degradation under laboratory conditions. The progress of degradation was monitored at intervals over 90 days by measurement of total gas evolution, VFA and ammonia production and by assessment of odour.

The experiments concluded that there was no evidence of nutrient deficiency and that, even with major changes, the presence of

potential inhibitors of the anaerobic reactions had no detectable effect on biodegradation rates or end-products.

The tests on higher dry substance lime showed that odour formation was not reduced and that increasing dry substance would not stabilise the lime. Indeed, this means of limiting the reactions would presumably only be effective at water contents sufficiently low for the dissolved solids to inhibit the micro-organisms through an osmotic pressure effect. In our experience, this does not occur even at 70% dry substance.

The remaining factors all showed significant effects and they are now considered in detail, with data obtained from factory lime pond operations. In the factory investigations, temperatures throughout the lime bed were measured at intervals over the storage period and the extent of biodegradation was monitored by analysis of VFA, pH and by simple odour ranking.

Soluble C.O.D. and Sugar Content.

Laboratory experiments on soluble COD and sugar content.

This experiment was designed to demonstrate the effect of reduction of the soluble C.O.D. of lime on biodegradation rate and on end-products. Portions of a carbonatation mud were filtered and washed with water to reduce the soluble solids content to 50% , 10% and 5% of that of the original material. Then 200 cm^3 samples of the washed muds and of the untreated mud, used as a control, were each inoculated with 2cm^3 of an inoculum prepared from active lime pond mud and incubated at 28°C under anaerobic conditions for 90 days.

At intervals, the gas pressure in the anaerobic vessel was measured and mud samples were extracted under anaerobic conditions. The vessel was then vented to restore atmospheric pressure. The cumulative total pressure generated over the 90 days was calculated. Mud samples were analysed for VFA and ammonia, and sample odour was assessed in descriptive terms and by rank of offensiveness. The ranking system used pooled data from this and other similar experiments to give a ranking scale of 1 (least offensive) to 10 (most offensive). The results are summarised in Table 2.

The results show that reduction of the soluble COD content of carbonatation mud reduced the products of biodegradation and odour, dependent upon the extent of the COD reduction. The material used in this experiment had a high sugar content of 1.2% on sample; reducing this to 0.6% sugar gave a marked reduction of bioactivity and foul odour, but a concentration of around 0.05% sugar was required to give negligible odour. This is unlikely to be achievable through Oliver filter sweetening-off practice, and an

additional re-washing process would probably be needed to achieve zero odour.

Factory Lime and Odour.

The major fraction of soluble COD is due to the sugar content of the carbonatation mud and therefore a relation between the sugar content and lime odour would be expected. To confirm this, Table 3 shows the factory 91/92 campaign mean % sugar in lime and sample odour rankings averaged over the period November 91 to April 92. A different ranking system from that used in the above section (Table 2) was used in all of the factory assessments, in which a scale 1 to 5 was used to denote increasing complexity and intensity of odour.

There is a clear trend to higher odour ranking with higher sugar content.   The relationship is not entirely consistent but other factors have been identified, as discussed below, which also affect odour rank so a simple direct correlation between sugar and odour would be unlikely.

Temperature

Micro-organisms operate under a wide variety of temperatures although optimum temperature conditions for growth of different organisms will vary. In a complex system, a particular tempera- ture will favour growth of some organisms whilst others will decline, so variations in lime bed temperature may lead to different end-products and reaction rates, according to the sensitivities of the groups of organisms present. Temperatures in lime ponds have been found to range from about 10 to 40°C, dependent on factory, time of year and sample point.

Laboratory experiments on temperature

The effect of temperature on carbonatation mud biodegradation and odour formation was investigated under anaerobic conditions at temperatures ranging from 10 to 40°C, using a procedure similar to that outlined in Section 4.2.1. Results are summarised in Methane production was slow at all temperatures but increased with the incubation temperature, indicating that biological activity increased with temperature. Total VFA concentration was high compared with methane evolved, indicating that the anaerobic pathway was virtually terminated following acid formation.   High VFA concentrations were reached in relatively short periods at temperatures in the range 25 to 40°C . At the lower temperatures of 10 and 15°C, VFA concentrations were moderate for up to 11

weeks but the concentrations reached indicate significant activity at these temperatures.

Lime pH at all temperatures fell to around neutral but at different rates. In the higher temperature range the lime started to fall from its initial value of pH 11.2 within the first week, whilst in the lower temperature range pH stability was maintained for 2 to 4 weeks.

The largest temperature effect was found in odour production, where strong and foul multiple-character odours were produced within the first week at the higher temperatures, whilst at 10 and 15°C odours were almost unchanged over a 12 week period.

The results indicate that biological activity decreases with reducing temperature and that the anaerobic pathway virtually stops after the acidogenic stage at all temperatures. Odour production in the 10 to 15°C range is low even though there is evidence of biological activity.

Factory observations on temperature effects.

Temperatures throughout the lime bed depth were measured at all factories over the storage period for the 91/92 campaign. They ranged from 8 to 34°C, with average values, excluding the immediate surface layer, of between 9 and 28°C. Over the campaign period there was little change, but temperatures during the summer indicated that the ponds had stabilised at between 15 and 20°C. Mean lime bed temperatures and odour rankings up to April 92 are shown in Table 5.

With the exception of 2 ponds at factory 6, odour rankings correspond well with lime bed temperatures.

At that factory, the lime feed was alternated periodically between three ponds to distribute it in thin layers to achieve additional cooling and partial drainage during the campaign. This distribution may have influenced the odour rank, particularly if there had been any aerobic activity in the lime layers.

The factory results support the laboratory conclusion that low temperature results in low lime odour. Lime bed temperature would be controlled by the temperature of the lime feed, by the heat generated within the bed by fermentation, and by the dissipation of heat from the bed. Significant correlations of odour rank and feed temperature or factors related to heat dissipation were not obtained from the available data, although factories with the lowest and highest lime feed and bed temperatures had, respectively, the lowest and highest odour ranks.

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pH.

Micro-organisms are sensitive to pH, and optimum conditions for growth vary, so in a complex system of organisms, end-products and reaction rates are very dependent on pH.

Experience of waste water management has shown the optimum to be between pH 7 and   pH 8 and that above pH 8.5 there is poor digestion and malodour. Lime at discharge to the ponds is between pH 11.0 and pH 11.5. This is too high for many organisms to grow, and so, by analogy with water treatment, the possibility of reduction of the pH by artificial means to a range more favourable for anaerobic digestion was considered.

Laboratory experiments on pH

Anaerobic digestion tests were undertaken with lime samples which had been treated with carbon dioxide to give initial pH 7.5, 8.5 and 9.5. The pH was not controlled but allowed to fall freely once the incubation had started.

No significant differences were found in odour and biological activity between an untreated control at pH 11.2 and the treated samples, and it was concluded that reduction of the starting pH did not materially influence the digestion process and odour production.

Factory observations on effect of pH

Lime pH values at all factories for the 91/92 campaign up to April 92 are summarised in Table 6 with odour rankings, first carbonatation pH and mean volatile aliphatic acid contents.

VFA concentrations were variable but in some lime ponds reached high levels, comparable to those referred to in Section 3, and then they are responsible for the low pH of the lime. pH conditions in the lime ponds at each factory became established early in the campaign, and so lime bed pH values did not deviate far from the reported mean values over the period to April 92. Odour rankings in this period correlate with lime pH reasonably closely, and it is apparent that high pH limits odour.   .

During the subsequent 3-4 month period until excavation in July/August 92, the lime at all sites dropped to between pH 7 and pH 8.5 and odours increased. It was noticeable, however, that at those sites where pH had remained fairly high and stable for most of the storage period, the odours did not exceed ranking 3. This difference in degradation pattern is illustrated in Figure 2, using

data for factories 1 and 3 which show the two patterns particularly clearly.

The probable explanation of this difference is the extent of methanogenesis. In the one case acidogenesis proceeds rapidly, VFAs accumulate, pH falls, methanogenesis becomes blocked and other organisms take over, creating malodours. In the other case, for reasons that are not apparent from these data, it is plausible that the rate of production of VFAs and the fall in pH are not sufficient to inhibit methanogenesis, so that good anaerobic digestion conditions prevail for many months, keeping a fairly steady VFA concentration. When acidogenesis accelerates in the warmer months, the mechanism of degradation moves towards the more odiferous one.

Apart from factory 6, which operated a distributed lime system (referred to above), odour rank correlates with first carbonatation pH and this suggests that this parameter has a stabilising effect. The buffer capacities of fresh lime cake from all factories were examined to establish if differences would account for the apparent differences in lime stability at high first carbonatation pH. Buffer curves were found to be very similar at all factories; curves for two factories are shown in Figure 3.

Buffer capacity is available at the upper end of the first carbonatation pH range but below pH 11.0 buffer capacity is low and therefore pH will fall rapidly with small increases in acid production. For example, the buffer capacity between pH 12.0 and pH 11.5 is about 2 g.eq/ kg, but 0.3 g.eq/kg between pH 11.5 and pH 11.0 and only 0.06 g.eq/kg between pH 11.0 and pH 10.5.

The production of, for example, 0.2 gram equivalent of acid/kg (e.g. 12g acetic acid/kg) would result in a drop to pH 11.2 at a factory with first carbonatation lime pH 11.45 and to pH 10.5 at a second factory with first carbonatation lime pH 11.1. As the slope of the buffer curve steepens below pH 11, further acid would accelerate the fall towards pH 7, with accompanying increased odour development.

Small differences are detectable in the buffer capacity between factories, but differences in first carbonatation pH would seem to have a bigger effect on the fall of pH during anaerobic degradation of the carbonatation mud. In practice, first carbonatation pH is likely to be governed by other factors but the results indicate that some stabilisation of lime may be achieved by maintaining high first carbonatation pH.

Additives

Two possibilities for control of biodegradation, use of biocides and addition of calcium oxide to increase pH, were tested in laboratory trials.

Biocides

Evaluations on lime were conducted by a biocide marketing company to establish suitable biocides for trial (2). Two commercial biocides, Nalco D4007 and 1115 used in combination, were recommended for test using the laboratory anaerobic test methods described above. In the test no significant odour production was detected for a period of nine weeks and then a marginal increase in odour occurred. The lime fell to about pH 9.5 and VFAs were produced during the incubation.

The use of biocide is therefore considered to have technical potential in lime odour control, but the uncertainties associated with possible residual effects have militated against further trials at present.

Calcium oxide

Observations on the possible effect of first carbonatation pH on lime stability suggested that artificially increasing lime pH and alkalinity with calcium oxide may be effective in carbonatation mud stabilisation, which would have an advantage over biocides in that no significant change in the lime constituents would result. Tests were conducted at addition levels of 0.5 and 1% CaO on lime. Addition of 1% CaO stabilised the lime without significant odour deterioration for a period of 5 weeks. Further work to establish the addition required to stabilise lime for longer periods is in progress.

DISCUSSION

Carbonatation mud contains organic materials including sugar, which contributes most of the soluble C.O.D., proteins, polysaccharides and calcium salts of organic acids. Storage in the traditional lagooning process leads, in the absence of aeration, to anaerobic breakdown of the organic content and this breakdown may result in malodour generation. Differences in the nature and intensities of odours at different factories and in different years are well known, suggesting that the progress of degradation is variable.

Analysis of stored lime from one factory, which had a recent history of odour problems, revealed that there was no significant loss of C.O.D over a period of 8 months. High concentrations of lower volatile aliphatic acids, principally acetic but including propionic and n-butyric acids, built up rapidly, resulting in a fall in pH until stabilised at approximately pH 7 due to the calcium carbonate content of the mud. Volatile acid odours were not detected as the free acid concentrations at that pH were low enough to be undetected (or possibly were masked by other odours). The stored lime developed foul odours, mainly of sulphide, indole, thiols and methylamines. These compounds were present at low but measurable concentrations which, unlike the volatile acids, remained fairly consistent during storage.

The conditions identified above are consistent with anaerobic degradation of organic material in which the metabolic pathway is terminated prior to the final stage. In this stage, acetic acid, formed by breakdown of more complex components, is converted into odourless products methane and carbon dioxide. Bacteria which facilitate this final conversion stage are known to be very sensitive to stress and are inhibited by high concentrations of fatty acids. If the conditions allow the rapid formation of high concentrations of VFAs from simple molecules such sugar, which is readily available in carbonatation mud at concentrations of about 10g/kg, then methanogenesis is inhibited, carbon is not removed from the system and volatile acids accumulate further, resulting in reduction of pH. If calcium carbonate was not present to limit the fall in pH, conditions would become acid and allow release of VFA odours. If hydrogen, produced at the acid-forming stage, is not utilised by methanogenic bacteria, it becomes available to sulphate-reducing bacteria, with the formation of hydrogen sulphide. Other secondary odour-forming reactions are also believed to be facilitated under these conditions, and so complex foul odours result. This, then, appears to be the mechanism operating in lime lagoons and it is probable that the availability of sugar from the lime facilitates the generation of the malodour-forming conditions.

The process as described does not take place to the same extent in all factory lagoons, as the lime at some sites was found to have a relatively high pH, moderate VFA concentrations and comparatively fresh odours after a long storage periods. This suggested that biodegradation may either be reduced or stopped, or take a more favourable course, in some situations.

Anaerobic degradation is a complex process and its course may be influenced by many factors. Some factors which were known to be significant in biodegradation were investigated in the laboratory to ascertain if control is possible, either by prevention or modification of the degradation. The results identified soluble C.O.D.content (mainly sucrose), pH and temperature as having

significant effects on odour production. Addition of calcium oxide or biocide has been shown to delay odour production, although conditions for complete stabilisation using these materials have not been established. Other factors such as nutrient availability or the effect of potential inhibitors of anaerobic degradation were not identified as significant factors.

In factory lime ponds, the effects of soluble C.O.D. (sucrose) content, pH of first carbonatation, and temperature and pH in lime ponds have all been shown to have significant effect on the lime odour. Odours were minimised by conditions which stopped or significantly reduced biodegradation.

Table 7 compares mean data for 91/92 stored lime at two factories, to illustrate the above observations linking odour to a number of operational parameters.

CONCLUSIONS

Storage of carbonatation mud over a 6-10 month period in the traditional lagooning process leads, in the absence of aeration, to anaerobic breakdown of the organic content. The progress of degradation is variable and may lead to malodours, and hence to environmental concerns.

Malodours are caused mainly by sulphide, indoles and thiols. Methylamines, especially trimethylamine, may contribute. Odours from volatile aliphatic acids are not usually discernible, presumably because they are present as calcium salts.

Anaerobic degradation with pronounced malodour production is characterised by a drop in lime pH to values below pH 9 and by volatile aliphatic acid contents of around 20 g/kg lime. It would seem that the desirable anaerobic pathway of methanogenesis leading to conversion of VFAs to odourless products is blocked at around pH 9. Because of the low buffer capacity of the lime below about pH11, VFA production causes a rapid drop in pH, although the lime does not fall below pH 7 because of the neutralising effect of its calcium carbonate.

Reduction of the soluble organic content of carbonatation mud to values corresponding to less than 0.1% sugar on lime are needed to prevent malodours, although concentrations of less than about 0.5% sugar are beneficial.

Temperatures below 20°C markedly reduce malodours, because of their retarding effect on micro-organism activity.

A first carbonatation end-point closer to pH 11.5 than to pH 11.0 restricts malodour production, again presumably because of its

adverse effect on micro-organism viability. The greater buffer capacity of the lime at the higher pH is also probably beneficial.

Adjusting the initial lime pH to around pH 8.5 seems not to alter the anaerobic pathway beneficially.

Raising the dry matter content, even above 65-70% solids, does not prevent undesirable anaerobic degradation.

Regulating nutrient availability or adding specific inhibitors of anaerobic activity has shown little promise.

Conditions occasionally prevail where the desired anaerobic course is apparently followed for months, but the means to achieve this in a controlled fashion have not yet been identified.

Preventing any anaerobic activity seems a practical way of stopping malodour production. Some biocides may be suitable, or, and probably simpler and cheaper, the admixture of small amounts of milk of lime to maintain the lime at above pH 11.5.

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Table 1. 1990/91 Lime Composition: Factories A and B.

		Lime Surface (<0.3m)				Lime Bed (0.5-4m)			
Week Number		1	3/9	11/15	18/35	14	24	31	35
	Site								
pH	A	11.0	10.6	9.9	7.5	7.5	7.3	7.4	7.6
	B	11.2	9.6	10.7	8.4			9.8	
VFA (g/kg)	A	0.11	1.72	5.70	13.92	13.80	13.30	22.30	6.80
	B	0.13	1.98	3.23	9.84			4.16	
C.O.D. (g/kg)	A	78.9	89.9	87.7	107.4	88.6	90.7	87.5	109
	B	66.5	82.9	81.6	73.4			98.5	
H_2S (mg/kg)	A	0.37	0.46	0.28	0.43	1.20	0.42	2.25	0.05
	B	0.33	0.75	0.04	0.22			0.04	
Thiols (mg/kg)	A		0.11	0.18			1.02	2.95	
	B								
Indoles (mg/kg)	A	0.03	0.02	0.02	0.06	0.24	0.17	0.18	0.03
	B	0.01	0.03	0.02	0.04			0.02	
Amines(mg/kg)	A	1.7	8.6	7.6	17.3	29.0	23.3	5.8	3.5
	B	0.5	5.2	13.3	25.2			31.3	

Table 2. Effect of Reduction of Soluble Organic Content.

Parameter	Sample			
	5% of COD	10% of COD	50% of COD	Control
Temperature(°C)	28	28	28	28
Sugar (%)	0.06	0.12	0.6	1.2
Cumulative gas pressure (bar, 90 days)	0.18	0.20	0.29	0.91
Total VFA (g/kg)	2.2	7.8	12. 4	20.7
Ammonia (mg/kg)	0.5	1.0	1.0	1.0
Odour rank	1	2	3	6
Odour type at 90 days	lime, sweet, estery	faecal	faecal	strong faecal

Table 3. Effect of Sugar Content of Factory Lime on Odour

Factory	Mean sugar in lime (%)	Mean odour rank	Odour description
1	0. 3	1.5	fresh mud, beety, ammonia
2	0.7	1.5	
7	0.9	2.5	slightly changed odour
6	0.6	2.5	
8	1.1	2.5	
9	2.0	3	weak foul odour
10	1.3	3	
5	1.3	4	strong foul odour
4	1.0	5	multiple strong foul odours
3	1.0	5	

Table 4. Effect of Temperature

Parameter	Incubation Temperatures (°C)					
	40	30	25	20	15	10
Methane (g/kg)	0.27	0.17	0.20	0.28	0.04	0.05
Time (weeks)	12	12	12	12	12	12
Total VFA (g/kg)	26.0	12.5	15.0	13.0	5.0	6.0
Time (weeks)	5	8	8	11	11	11
Time above pH 11 (weeks)	0.5	0.5	0.5	1	4	2
Odour rank	5	5	5	5	1.5	1
Time (weeks)	0.5	2	6-9	11	12	12

Table 5. Factory Lime Temperatures and Odour

Factory/pond	Lime Bed mean temperature (°C)	Mean odour rank	Feed temperature (°C)
1A	9.5	1.5	25
1B	13.8	1.5	25
8	15.6	2.5	
6A	16.9	2.5	36
7	17.5	2.5	
2	17.6	1.5	36
5	17.8	4	38.5
4	18.6	5	32.9
10	18.9	3	36
9	19.9	3	38
3B	20.0	5	44
6B	20.1	2.5	36
6C	21.7	2.5	36
3A	27.4	5	44

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Table 6. Factory Lime pH and Odour

Factory/ pond	Lime Bed mean pH	Lime odour rank	First carbonatation pH	Mean total VFA (g/kg)
1A	11.5	1	11.5	2.0
1B	11.0	1.5	11.5	2.0
2	10.8	1.5	11.4	1.6
7	10.6	2.5	11.3	1.5
6A	10.2	2.5	11.5	4.3
8	10.1	2.5	11.4	3.5
6B	10.0	2.5	11.5	4.3
9	9.7	3.0	11.3	10.2
6C	9.6	2.5	11.5	4.3
5	9.4	4	11.1	5.1
3B	9.4	3	11.1	10.7
10	9.3	3	11.3	4.9.
4	8.6	5	11.0	8.3
3A	6.9	5	11.1	10.7

Table 7. Comparison of Low and High Lime Odour Factories

	Factory 1	Factory 3
Sugar in lime (%)	0.29	1.01
Lime feed temperature (°C)	25	44
Lime bed temperature, Nov 91 to April 92 (°C)	14	27
Lime bed temperature April 92 to August 92 (°C)	19-22	17-23
Lime feed pH	11.5	11.1
Lime bed pH	11.0	7-7.4 throughout
Lime bed pH started to fall	June 92	
VFA (mg/kg)	mean 2.0, max 5.5	mean 10.0, max 20.0
Odour ranking	1.5 to June 92, reached 3 in July 92	5 throughout
Odour description	Beety, ammonia, sweet, becoming weak foul in July 92	Foul, faecal, H_2S, thiol, amine throughout

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FIGURE 1 - DESCRIPTION OF ANAEROBIC PROCESSES (Banks, 1992)
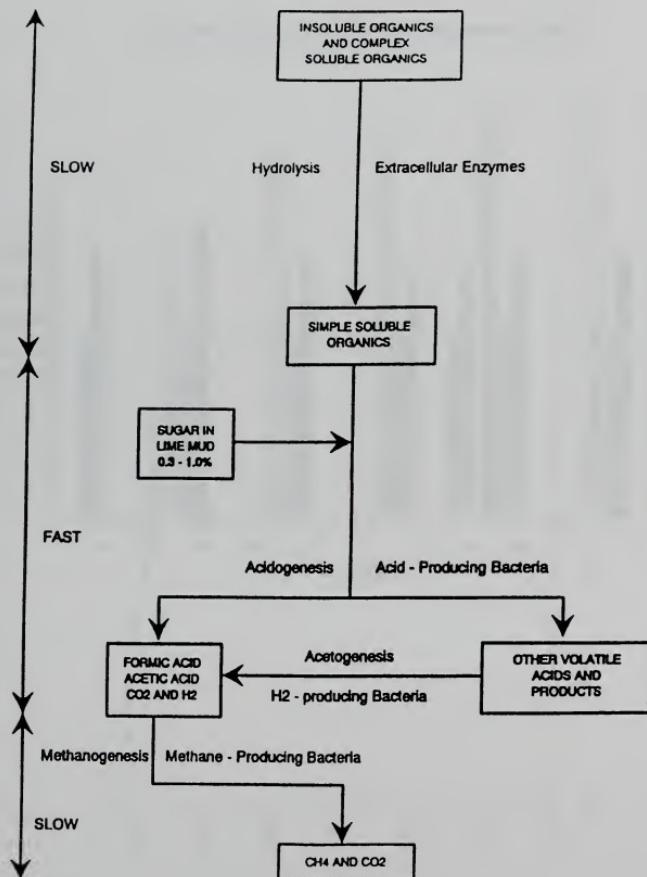
Figure 2 - Comparison of High and Low Odour Factories
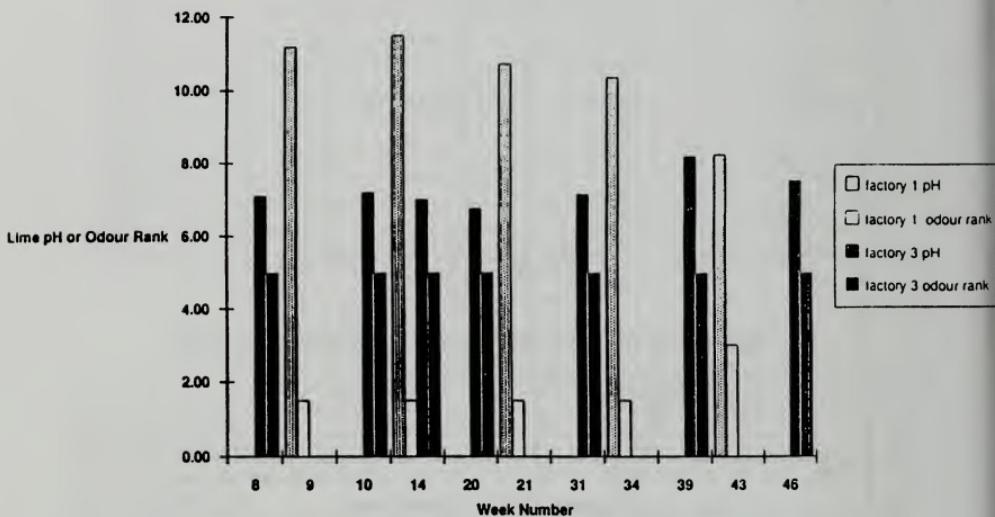
Figure 3 - Factory Lime Titration Curves
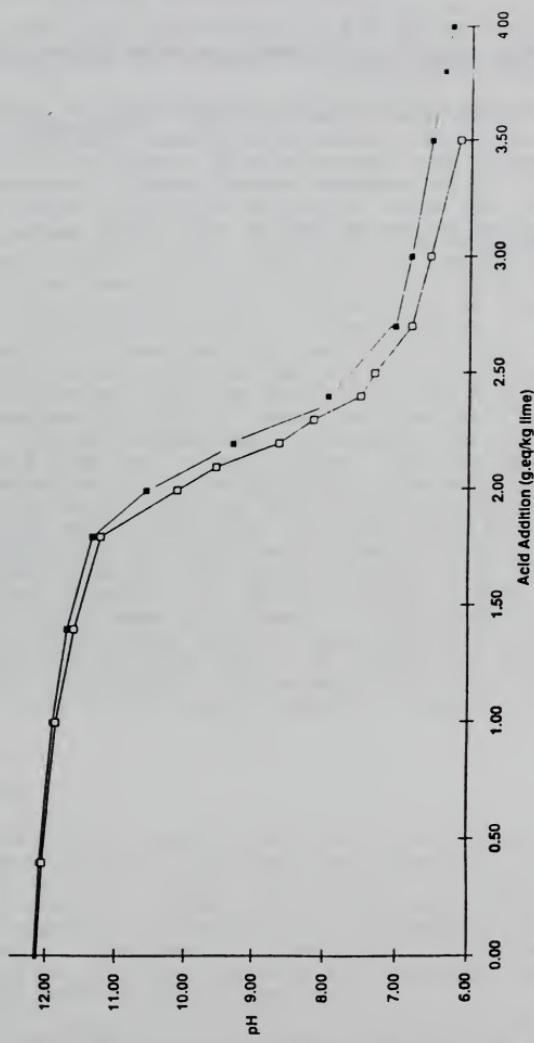
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DISCUSSION

Question: Working in the U.S., we certainly are aware of our
legal environment. Could you comment on some aspects of the
legal environment in the U.K.?

Parslow: Yes. There are no defined odor limits. Basically, if
the local authority, or a group of citizens, can prove that an
odor is a nuisance, then you have to do something to abate the
odor situation. If the company cannot supply a reasonable method
to do this, then the local authority may supply some "advice" or
require certain actions which may or may not be useful. It's a
very uncertain area.

DRYING OF BEET PULP IN SUPERHEATED STEAM UNDER PRESSURE

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ABSTRACT

A new method for drying beet pulp has been developed in Denmark,
and full-scale plants are now operating in Denmark and France. The
pressed pulp is conveyed into a modified cellular fluid bed under
pressure.   The particles are carried by a vertical superheated
steam flow circulated within a closed vessel.   In the cells,
intimate contact is achieved between the particles and the super-
heated steam, and at the same time the particles are conveyed from
the first to the last cell in such a way that the small particles
with the shortest drying time reach the last cell before the large
particles.   The steam developed by evaporation from the particles
is discharged from the closed system.   This steam of, e.g. 3 bar
gauge, is used for juice evaporation.   The method offers 3 major
advantages: 1) Energy saving: The energy supplies leave the drier
as steam in an applicable form.   By using this steam, much of the
energy is recovered and more than 90% may be saved compared to the
amount of fuel used by other drying plants, e.g. drum driers.   2)
No pollution: Contrary to other drying methods, there is no
emission as the system is closed.   3) Improved product quality:
Because the drying takes place without admission of air, there is
no oxidation and material loss.   No impurities from flue gas can
mix with the dried pulp.   Furthermore, the heat treatment of the
beet pulp when dried under pressure improves its digestible
qualities.

INTRODUCTION

Drying of beet pulp in sugar factories is very energy-consuming and
causes severe pollution problems when conducted in directly fuel-
fired rotating drum dryers. If instead the drying is done in
superheated steam in a closed system, air pollution can be avoided,
and energy recovery is possible. If furthermore, the pulp is dried
under pressure, the possibilities for energy recovery are even
greater, and the increased density of the steam improves the heat
transfer and reduces the drying time.

This paper describes the development and function of the pressur-
ized fluid bed dryer over the last 10 years and its implementation
in a sugar factory. The dryer has the following main advantages:

* Energy saving
* No pollution
* Improved product quality

History

The development of the DDS steam dryer started at one of the Danish
Sugar Corporation's factories at Stege. In 1978 the first ideas
took form. Small fluid beds, where pulp was fluidized by air, were
built in order to examine the special features of the process of
fluidizing beet pulp which cannot be fluidized in a conventional
fluid bed because of the pulp structure. However, due to other
urgent jobs, the development was temporarily stopped for 2 years.

In 1981 a group of three engineers was established. Further lab-
scale fluid beds were examined, and a single-cell fluid bed with
superheated steam under pressure was built in 1982. The steam used
came directly from a low-pressure boiler, and it was not recir-
culated. In that model it was possible to dry pulp in batches, and
various measurements necessary for proceeding to the next step were
taken.

In 1983 a pilot plant was built at Stege Sugar Factory (see Figure
1).

The fluid bed was divided into 16 cells in order to obtain the
necessary retention time. The steam leaving the dryer was first
cleaned of dust in a cyclone, then scrubbed with water, before it
was reheated and returned to the fluid bed by means of a fan (see
Figure 2). The various components were connected by pipes forming
a closed cycle system, which could withstand a pressure of 4 bar.

During the 1983 sugar beet season and the 1984 off-season, the dust
separating system in the top of the fluid bed was improved, so that
the plant could be simplified by removing the external dust
separation systems. The plant then looked as shown in Figure 3.

The plant had a drying capacity of 1,000 kg H_2O evaporation per
hour. After a series of measurements during a successful season in
1984, it was decided to build the first full-scale plant. The idea
was to place the heat exchanger in the centre of the fluid bed and
the fan at the bottom, so that the big circuit of steam and all
components were contained in one pressure vessel.

In 1985 the first full-scale plant was ready for operation at Stege
Sugar Factory in Denmark. The capacity was 6 t/h water evaporation.
In the following years this plant was further developed to obtain
a capacity of almost 9 t/h evaporation.

1987-88 was nominated the European year of the Environment by the
European Council of Ministers. In this connection the dryer was
awarded a prize presented by His Royal Highness Prince Henrik of
Denmark in his capacity as patron of the World Wildlife Fund (WWF).

In 1990 a DDS steam dryer with an evaporative capacity of 25 t/h was erected at Nangis Sugar Factory in France. In March 1991 this plant was awarded an environment prize at a special ceremony in the Eifel Tower by the French organization APAVE responsible for the environment at French companies.

The further development of the steam dryer is handled by NIRO, a company in the DANISCO Group. NIRO-DDS, a subsidiary of NIRO, is responsible for marketing the DDS steam dryer to the international sugar industry. The rising market demand for non-polluting technologies and processes has also been felt in NIRO-DDS, which has received numerous enquiries and already concluded five contracts for DDS steam dryers this year.

OPERATION: How the DDS steam dryer works

The pulp is dried in a cell-divided fluid bed carried by a vertical flow of superheated steam circulated within a vessel under pressure.

Figure 4 shows an open view of the dryer. The pulp is introduced by the screw conveyer (1) into the first of the 16 cells placed around the superheater (7) in the centre. The impeller (8), which is the only moving part of the dryer, blows the superheated steam up through the perforated plates at the bottom of the cells.

Figure 5 shows one of the cells. The beet pulp is kept moving by the flow of superheated steam coming from below. In the lower part of the cell, the horizontal cross-sectional area has been reduced by filling in the triangular body shown. This results in higher average velocity of the steam; in this way large particles will also be kept moving. The triangle also creates a rotating movement of the material, and all large particles will thus find their way to the opening (a) of the next cell. The light particles will be lifted to the upper part of the cell, where the inclined plates (b) distribute the flow of steam in the large cross-sectional area. The pulp particles will fall down on the upper sides of the plates and slide downwards on them. Some of the particles will be guided by the rails (c) towards the opening (d) to the next cell. In this way, the pulp particles will finally reach cell 16, from where they are carried away by the screw conveyor (10). A proper design of the rails (c) and the bottom part of the cells results in a prolonged retention time of the large particles compared to that of the small ones, corresponding to the difference in drying time.

The steam that passes up through the cells to the top of the dryer will contain some dust particles (smaller than 1 mm). This dust-filled steam passes between the stationary blades (2) creating a vortex in the cylinder limited by the wall (3). The dust collects

on the wall and passes through a slot into the side cyclone (4), from where it is sucked into cell 16 by the ejector (5).

The dustfree steam passes between the stationary set of blades (6) forming some channels, in which the kinetic energy of the vortex is transformed into elevated pressure, which again helps to bring the steam down through the tubes of the superheater (7). During its passage down through the tubes the steam is superheated by steam of higher pressure condensing on the outer surface of the tubes. From the bottom of the apparatus the steam is again blown through the cells by the impeller (8), and the big circuit is closed. The steam evaporated from the beet pulp is discharged through the pipe (9) at the top. Since the steam is taken out at the centre of a vortex, the steam contains less than 10 ppm dust, which means it can be used in the juice evaporator without any risk of dust accumulation.

The energy supplied to the dryer is consequently recovered, and the air pollution known from drum dryers does not exist.

As drying takes place under pressure, the pulp must pass through a lock to enter the pressure system. This is done by means of a specially designed rotary valve placed in front of the screw conveyer, (see (1) in Figure 4). The rotary valve is shown in Figure 6. The rotor has three chambers, between which there is a relatively long distance on the cylindrical surface in order to minimize steam leaks. On the two sides there are ajustable bronze blocks. A chamber loaded with beet pulp is filled with steam through the pipe shown and put under pressure, before it is emptied downwards. This steam is taken from the bottom of the dryer after the impeller. The steam heats the pulp to saturation temperature, builds pressure in the chamber and helps to blow the chamber clean. As the empty chamber is turning upwards again, it is released from pressure through another pipe, before it is ready to be filled again. This steam exhaust is used for preheating the pulp in the screw conveyer feeding the rotary valve.

Similar to the inlet rotary valve, there is an outlet rotary valve without steam pipes through the ajustable bronze blocks. The dried pulp and steam are separated in a cyclone, from which the steam is also conducted to the screw conveyer in front of the inlet rotary valve.

Consequently all waste steam is recycled and reused in the system.

DRYER CAPACITY AND CONTROL

The temperature of the circulating steam after the superheater, (7) in Figure 4, depends on the pressure of the steam supplied. A high supply of steam pressure raises the temperature of the circulating

steam after the superheater. An increase in the temperature of the steam blown up into the cells raises the drying capacity.

The relation between supplied steam pressure and dryer capacity is shown in Figure 7, in which the capacities of three dryer sizes are indicated. Size 6 m (6 m diameter at the top) was the first plant in operation at Stege Sugar Factory in Denmark. Size 8 m was first supplied to France in 1990. Size 10 m will be put into operation in 1993.

The capacity curves apply for 2 to 3 bar gauge steam in the dryer. This pressure is also suitable for use in the evaporator in most sugar factories. With pressures higher than 3 bar g, the ∆t in the dryer, and thereby the capacity, is reduced. With less than 2 bar g pressure, the specific volume of the circulating steam increases, so that the circulating flow in t/h is reduced, which again reduces the capacity more than the increase achieved by the bigger ∆t.

The control of the pressure in the dryer is fairly simple. In a sugar factory the steam from the dryer is generally used in the first step of the evaporator. This means that the pressure in the dryer will follow the variations of pressure in the evaporator and through its volume stabilize the evaporator plant. To maintain the pressure in the dryer in case of a severe pressure drop in the evaporator, a pressure control valve could be installed between the dryer and the evaporator.

The superheat in the top of cell 15 is closely related to the dry substance of the dried material. This interdependence is used for controlling the drying process, either by regulating the pressure of the steam in the superheater or by regulating the flow of material supplied.

THE DDS STEAM DRYER AT NANGIS, FRANCE

After a visit at Stege Sugar Factory in the season of 1989, the factory management of Nangis Sugar Factory, owned by Lesaffre Frères S.A., decided to have a steam dryer installed for the 1990 season. Nangis had not previously dried its pulp, so the project included not only the dryer, but a complete plant with a transport system, granulation, pellet cooling, a silo for temporary storing and a new building. The evaporator was extended with one extra effect placed in front of the existing six effects. The time for implementing this comprehensive project was very short, but the plant was ready for the start of the season.The dryer erected at Nangis is a 8 m diameter DDS steam dryer with an evaporative capacity of 25 t/h, and supplied with 25 bar g steam. The mass and energy balance are shown in Figure 8.

At Nangis some tests were made with alkaline diffusion, during which the dry substance in the pulp increased to 38%. Under different operating conditions the dry substance content was only 22%. These tests provided the opportunity to prove that the dryer's evaporative capacity does not depend on the dry substance in the wet pulp, even within that wide range.

As mentioned before, the juice evaporator was extended from 6 to 7 effects. Figure 9 illustrates the steam system in a simplified diagram. After installation of the DDS steam dryer, Nangis obtained an extremely low fuel consumption of only 12 kg heavy fuel oil per 100 kg white sugar <u>including</u> steam drying. This is probably a world record. But as only 25 bar g steam is available at Nangis, and as part of the steam to the evaporator comes from the dryer, the factory cannot produce all the electric power necessary by itself. Some power has to be bought from the public grid. In spite of this cost, the total energy costs are still reduced taking into consideration the great fuel saving. This will also be the case outside France, even if electric power is more expensive than in France.

ENERGY FLOW AND ENERGY SAVING

Any thermal drying means transformation of energy. After drying, the energy used is available in a more or less usable form.

In drum drying, none of the energy supplied as fuel can be recovered. In order to evaporate 32 t/h in a drum dryer, you would need a fuel consumption of 26 MW is required, which is all lost, including the 6 to 10 MW that could have been transformed into electric power.

A steam dryer consumes almost the same amount of energy in order to evaporate 32 t/h, but about 90% of the energy leaves the dryer as steam which can be used in the first effect of the evaporator. Furthermore about 35 t/h of hot (140°C) condensate is available either for heating part of the thin juice flow, or for direct use in the boiler house as feed water. Consequently the introduction of steam drying hardly influences the fuel consumption in the boiler house. If for instance the steam dryer is supplied with 16 bar g steam, it will produce 3 bar g steam. This pressure reduction means that a possibility of transferring part of the energy into electric power is lost. The pressure difference from 16 to 3 bar g could have been used to produce 2 MW. It is worth to note that the 2 MW is not used by the dryer, but is still available after drying as usable heat in the form of low-pressure steam.

In other words drum drying with an evaporating capacity of 32 t/h requires 26 MW as fuel, but steam drying requires almost no extra

fuel to be supplied to the boiler house. However, a possibility of transforming 2 MW into electric power is lost.

The energy flow in a sugar factory can be illustrated as in Figures 10A and B. In this example the sugar factory has a capacity of 6,000 t beet per day. The beet pulp is pressed to 28% DS which corresponds to an evaporating capacity of 32 t/h in the drying plant.

Figure 10A shows a fairly energy efficient sugar factory. The fuel consumption is 77 MW, of which 26 MW is consumed by the drum drying plant and 51 MW goes to the boilers. Assuming a 40 bar g steam pressure, 7 MW power is generated and 39 MW goes to the evaporator as 3 bar g steam. This 39 MW corresponds to 25% steam on beet.

Figure 10B shows the energy flow if the same 6,000 TBD factory introduced steam drying. Please note that the entire fuel supply to the drum dryer is saved and the boiler house has exactly the same size, but as part of the 40 bar steam flow is taken from the turbine at 12 bar g to supply the steam dryer, the power generation is reduced to 5.3 MW. This might be just sufficient for a sugar factory of the size in question.

If a greater power production is wanted, it can of course be obtained by increasing the boiler pressure. Figure 11 shows the relation between the power production and the live steam pressure on the basis of a steam system as illustrated in Figure 10B. The operating conditions of this system are as follows:

a)   Drying of all pulp
b)   Pulp pressed to 28% DS
c)   Factory size 6,000 TBD
d)   The evaporator supplied with 25% o.b. low-pressure steam.

On the same diagram the consumption of fuel including that of the steam dryer is indicated by dotted lines.

Great importance has always been attached to fuel saving, but a CO_2 tax on exhaust gas may attach even greater importance. The possibility of using beet pulp as fuel in a beet sugar factory is at hand. The prices of fodder may continue to go down, and beet pulp is considered to be duty-free, that is exempt from CO_2-tax. Dried pulp can be burned without emission of smell and can even be admixed with molasses before burning. With the introduction of steam drying, there will even be a surplus amount of pulp to be sold. Burning of beet pulp is not economically feasible today, but this might change in the future.

ADVANTAGES OF STEAM DRYING

Lack of pollution.

One of the greatest advantages of steam drying is that there is no environmental impact on the surroundings as is the case with drum drying. The steam from the dryer is condensed, so there is no emission to the atmosphere and consequently no pollution with dust or smell. The condensate formed by condensing the steam is suitable as fresh water in the diffuser after cooling in a heat exchanger with thin juice. It contains a little acetic acid (100-200 ppm) and a little dissolved ammonia (NH_3) (about 50 ppm), both of which are evaporated from the pulp in the dryer. The pH varies according to the content of ammonia. At Stege the pH varied between 7 and 8 and at Nangis between 7.5 and 8.5.

Improvement in product

When pulp is dried in its own superheated steam, no impurities from flue gas will be admixed, such as heavy metals and sulphur dioxide.

As there is no oxygen in the dryer, no oxidation of the product occurs, which means no loss of dry substance and no half burnt particles in the dried product. In drum dryers the loss of dry substance is between 3 and 10% depending on the load of the drum dryer (inlet temperature 600 to 1050°C).

In addition the feed value is improved by up to 20% according to tests made in the following way: In experiments at the Royal Danish Institute of Cattle, samples of steam-dried and drum-dried beet pulp in nylon nets have been injected directly into the cattle paunch. After various retention times the samples were taken out again, and the degradation of the solids as a function of the time was determined for pulp dried in the two different ways. Based on this information, the recovery of the product was calculated. Dairy cattle, with a short transition time through their digestive organs, will have an especially higher efficient yield of the feed which is steam dried (+20%).

Mixing of molasses for pellet formation

It is possible to admix molasses into the pulp before drying it in a DDS steam dryer. In addition, and contrary to the practice with drum dried pulp, large quantities of molasses can be admixed into the steam-dried pulp at the very moment the pulp is discharged from the dryer, because the pulp then has the following characteristics:

1. The product is very porous after having just been depressurized
2. All pores are filled with steam
3. The product has a temperature of 100°C

As an example, you can mix 50 kg of 77 Brix, 110°C, hot molasses can be mixed into 100 kg pulp dried to 91% dry substance. The molasses must be admixed at the very moment the pulp leaves the dryer and before air is admitted. After some seconds of intensive mixing, the pulp is slowly stirred for 10 minutes. During these 10 minutes the pulp is cooled by air at 70°C, pressed to pellets and then cooled again. The remaining heat will evaporate so much that the final product will have a dry solids content of 90%.

CONCLUSIONS

Special features of the DDS steam dryer
The DDS steam dryer is today fully developed and has proved its capability to dry beet pulp in a way which has many advantages compared to the conventional drying processes.

* High reliability
  The DDS steam dryer has demonstrated its ability to run a whole season without production stops caused by the dryer.

* Automatic operation
  The DDS steam dryer is easy to control; the operator can be located in a central control room away from the dryer.

* Compactness
  The DDS steam dryer requires very little space. Three steam dryers, type 8 m, need no more space than a drum dryer with 20 t/h evaporation.

* No fire risk
  As drying takes place without admission of air, there is no risk of fire.

* Low maintenance costs
  There is only one moving part – the impeller – in the DDS steam dryer. In the cells no measureable wear has been observed, even after 5 years.

* Low noise level
  Noise level is 70 dBA.

* No dust in the drying plant
  As drying takes place in a closed system, and the closed screw conveyors in the drying plant are vented backwards, the plant is totally free of dust.

As drying takes place in a closed system, and the closed screw conveyors in the drying plant are vented backwards, the plant is totally free of dust.

The three most important characteristics of the DDS steam dryers are:

1.   NO POLLUTION.   No emission of dust or odour, because the product is dried in a closed system. The condensate arising from condensation of steam is suitable for juice extraction.

2.   ENERGY SAVINGS.   More than 90% energy saving is possible by recover of the steam leaving the dryer and its use it in the evaporation plant.

3.   IMPROVED PRODUCT QUALITY CONTROL -. As the product is dryed without admission of air, any oxidation and loss of dry substance are avoided. In addition the product is not contaminated with flue gas impurities, and the heat treatment increases its digestibility.

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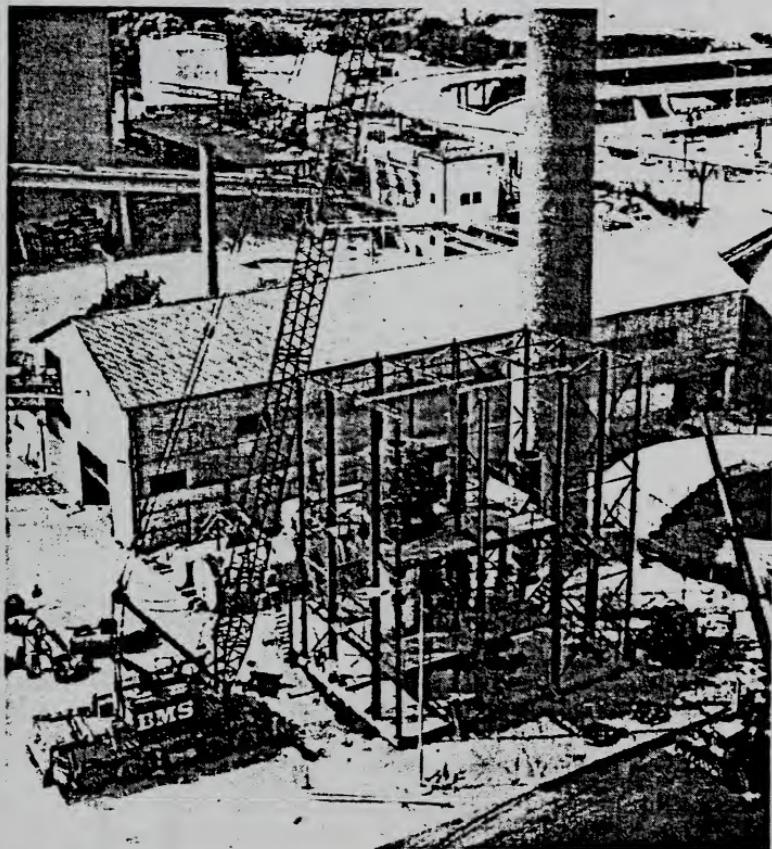
Fig. 1 Pilot plant under erection at Stege
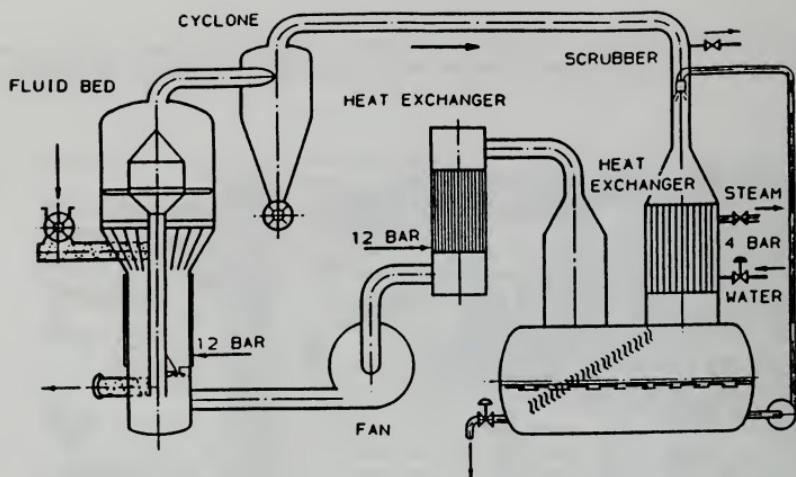
Fig. 2 Pilot plant complete 1983
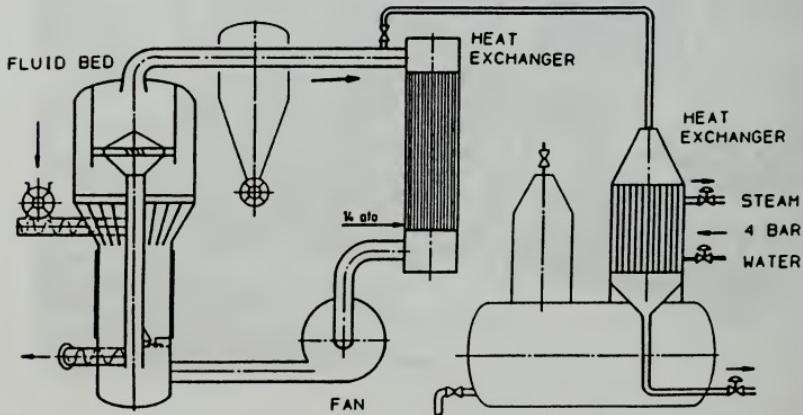
Fig. 3 Modified pilot plant 1984

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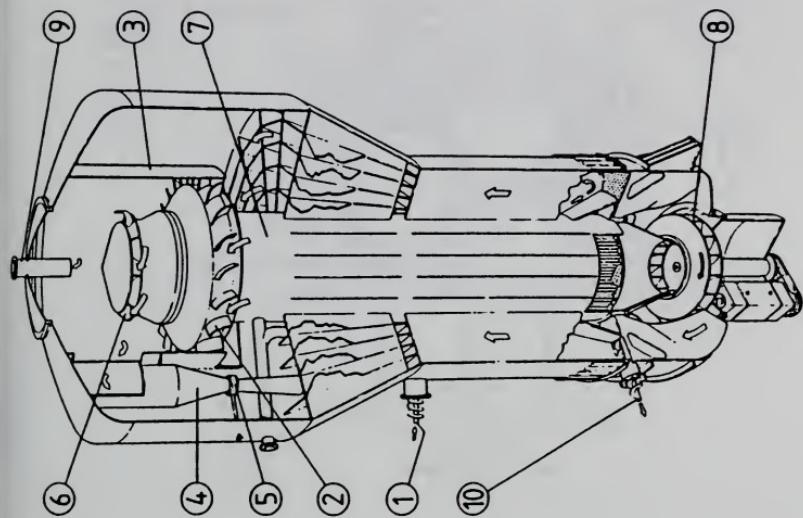
Fig. 4 Open view of an industrial scale dryer
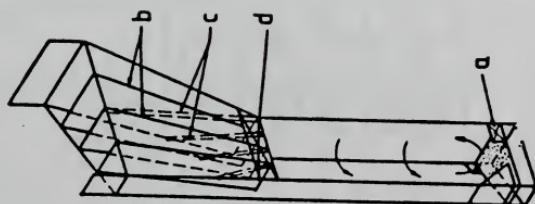
Fig. 5 Sectional view of one of the cells

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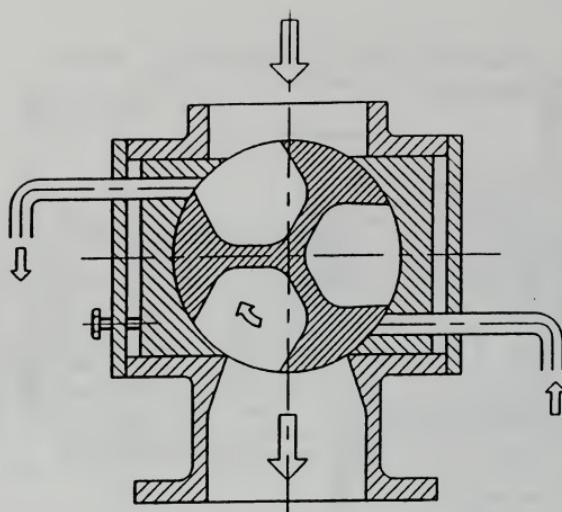
Fig. 6 Rotary valve
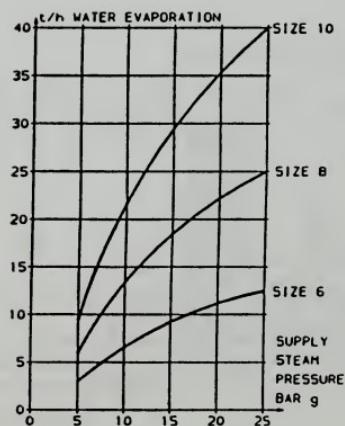
Fig. 7 Capacity diagram

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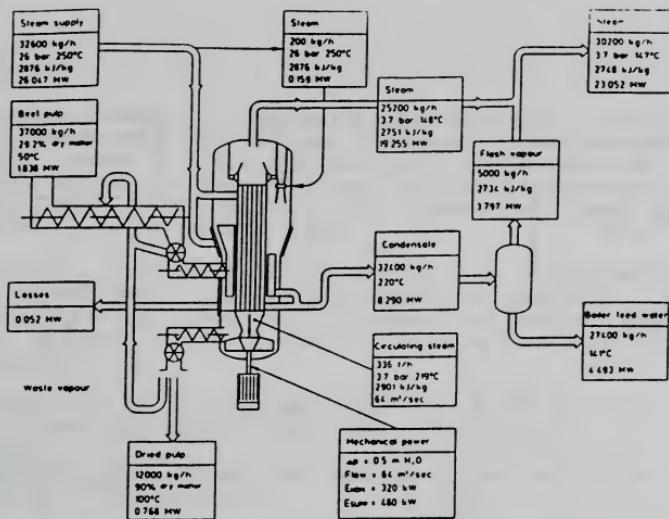
Fig. 8 Mass and energy balance for Nangis Sugar Factory
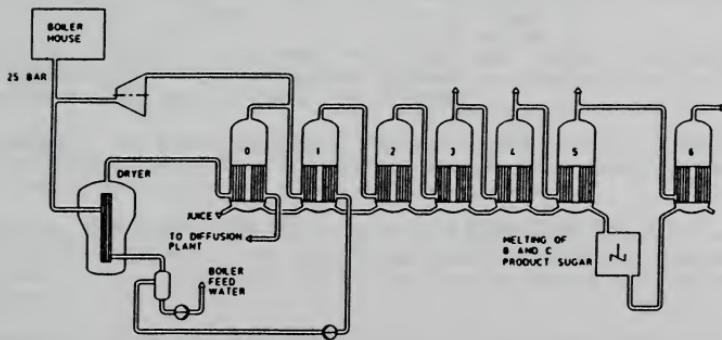
Fig. 9 Steam system at Nangis Sugar Factory
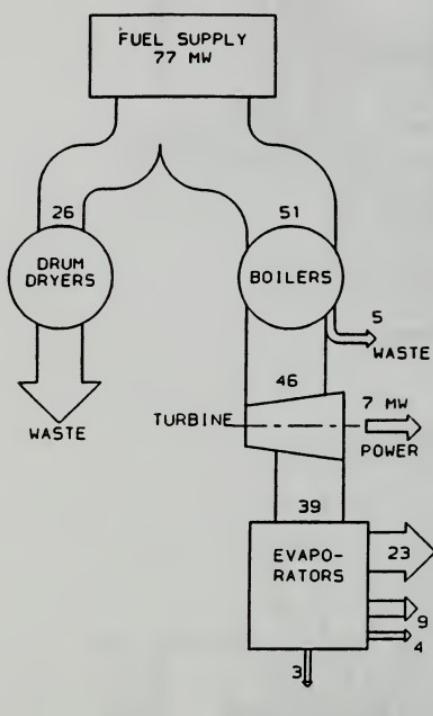
Fig. 10A Drum drying
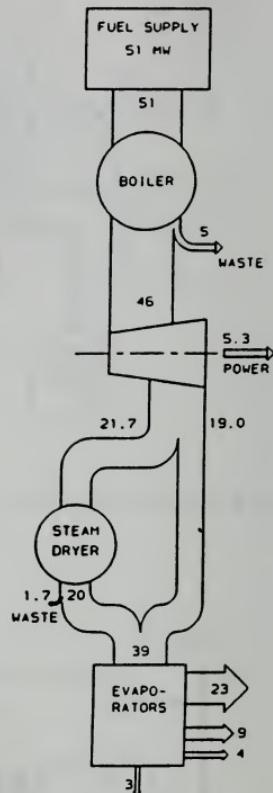
Fig. 10B Steam drying

DISCUSSION

Question: I want to congratulate you on a very fine paper. Your system should certainly become widespread and solve many problems. We have looked at this problem also, and calculated the energy savings compared to the investment and could not get a satisfactory payback time. Do you have any figures on costs compared to investment? or on payback time?

Andersson: I don't have the figures for energy savings, but I fully agree: the process cannot payback satisfactorily on the cost of energy saving alone. It's necessary to take the other environmental problems into consideration also before you can justify the installation.

Question: On an early slide, you noted 7 plants sold. Are these all for beet factories or for other materials?

Andersson: They are all for beet factories in Europe. There are actually 9 installed now.

Question: You mentioned that the larger particles have a longer retention time in this system. Do you have any information at all about how the particle size influences retention time in the drier?

Andersson: I don't have the time difference, but some tests have been made, using different colors, on beet pulp to measure retention time. It's been found that all particles with the same dry substance will have the same retention time, regardless of particle size.

Question: You've pointed out that you have a no-corrosion phenomena: that's right, because the system operates in the absence of oxygen. But do you have any erosion?

Andersson: The plant in Stege, Denmark has run for 5 seasons, and no erosion has been observed there. The plant at Nangis, now going into the third campaign, shows no erosion either.

Question: We are in the cane, not beet business, but I've enjoyed your paper very much. Two questions: what construction material do you use in the separator after the vents, where you might have erosion?

Andersson: It's not stainless steel. It's a hard material but I can't give you the exact composition. There is no sand at that point in the process - the sand will be at the bottom of the grid.

Question: Second question: you said there was no contamination from the steam from the first body of the multiple effect evaporators?

Andersson: I said there was no dust contamination. It is clear condensate from the first evaporator, containing a small amount of acetic acid.

Question: Is that condensate of good enough quality to be used as boiler feed water?

Andersson: No, it's not good enough for boiler feed, but it can be used in the extraction system.

Question: You mentioned the improved quality of the pulp produced in the steam drier. Do you have some figures on feed value compared to pulp produced at high temperatures?

Andersson: Only indications: one report from a Danish Institute showed digestibility to be 20% higher than with normal pulp. This data is supported by results from a French Institute showing 10% increase in digestibility.

A NEW CONCEPT OF LASER POLARIMETRY AT 650 AND 780 nm WAVELENGTHS

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ABSTRACT

A new method and a device is described for measuring optical rotation of sugar and other optically active solutions by using laser diode of LED light and timing measurements to evaluate optical rotation angles. The developed polarimeter measures the absolute angle of rotation of an optically active solution. Quartz plate calibration of the instrument is not needed. Measurements at 650 nm and 780 nm will be used for clear and dark solutions, respectively. The newly developed polarimeter is completely computer (PC) controlled and allows measuring times as short as 20 ms. Developed software allows for continuous process monitoring and everyday laboratory use through an user friendly and mouse driven interface.

INTRODUCTION

A polarimeter measures the optical rotation angle of the plane of polarization of a light beam as it travels through an optically active sample solution, e.g. a sugar solution. The angle of rotation is directly proportional to the sugar concentration in solution. At specified conditions (temperature, concentration and cell length) each optically active compound has its characteristic specific angle of rotation. The angle of rotation may be positive or negative.

In the manufacture of sugars, polarimetry is a widely used method to control sugar contents of process solutions at factory sites. Teaching, research and control laboratories also use polarimeters. All known commercial polarimeters use incoherent, filtered light sources with relatively low brightness and fairly high power consumption. Their spectral band widths range from a few nanometers to about 0.1 nm depending on the light source used. Recently incoherent, near infrared LED sources have been used for measuring dark optically active solutions (3). The band widths of LED sources in polarimeters are detemined by the interference filters used to choose the operation wavelength, typically 6nm. Using coherent,

high intensity, narrow-band laser light allows for penetration through dark solutions in cells even of 5 cm length. From the theoretical point of view one would like to use as narrow band light source as possible to enable accurate use of the wavelength dependence of the specific rotation, from instrument to instrument. For single mode lasers absolute wavelength accuracies of sub 0.01 nm, and half widths less than 0.01 nm, may be obtained. Recent availability of single mode diode lasers has made it possible to try these light sources in polarimeters. So far the only 'accepted' laser wavelength is the stabilized He-Ne wavelength of 632.9914 nm (2). Diode lasers offer small energy consumption, small size, high brightness and long lifetime, besides good wavelength stability and narrow band width of radiation under stabilized operation. There is a good selection of wavelengths available for single mode diode lasers, ranging roughly from 650 nm to 1500 nm. From practical reasons we have chosen to study lasers emitting at 650 and 780 nm. In our case lasers were actively wavelength stabilized. The majority of polarimetric laser measurements have been carried out in research laboratories. Several aspects of using coherent light in polarimetry have been discussed during the last few years (2).

Most commonly used polarimeters use Faraday compensation to measure optical rotation (2). The polarized light is directed through a sample cell and the rotation of the polarization of light is compensated by using a magneto-optical cell, where alternating current produces a compensating magnetic field bringing the direction of polarization of the light beam back to its original value. Compensation current is directly proportional to the angle of rotation. Compensation is limited to a few degrees. The compensation method is sensitive to any magnetic changes of the measuring environment, e.g. latitude. The instruments have to be calibrated at each location by using standard solutions or quartz plates. If lasers are used in Faraday type polarimeters, anisotropic effects have to be considered in the compensation process (2).

Known approaches of polarimetric technology are presented in the following publications (8,9). Commercial polarimeters use photomultiplier tubes to monitor light intensity variations of the polarized light. These devices need high-voltage power supplies and special care has to be taken in industrial environments to meet safety requirements.

Using lasers as light sources in polarimeters has been described earlier (1) and in two patent publications (6,7). In these studies, improved transmission of the light beam through solutions and slightly improved measuring accuracy due to the narrower band width have been reported.

The purpose of the present study was to develop a new polarimeter that will give the absolute angle of rotation of an optically

active material or solution with an accuracy of one thousandth of a degree or better, in the region, from -180 to +180 degrees.

The wavelengths chosen were adjusted for best performance in measuring clear (650 nm) and dark (780 nm) solutions, respectively. For dark solutions sample cells as long as 5 cm may be used. The new techniques allows measuring the absolute angle of rotation of an optically active solution each time. Accordingly, quartz plate calibration of the instrument is not needed, assuming the long term stability of the measuring wavelength is attained. The method used is completely insensitive to the magnetic field of the measuring environment. Computer control of the device allows storing extensive pre-measured data for easy operation in many different measuring situations, both simple and more demanding. Characteristic features of the method are presented below.

RESULTS AND DISCUSSION

The method differs from all of the previously reported methods in the way cross-polarization positions of the first polarizer and the rotating analyzer, with respect to each other, are obtained. The rising part of the oscillating signal is used for triggering; the time delay between the measuring and the reference beams is measured and, simultaneously the time span of the full period of the reference beam is measured.

In most earlier methods the minimum light intensity critetia has been used to observe the cross-polarization position of the analyzer and the polarizer. For measuring time differences a crystal oscillator and an electronic counter are used. The present method allows using simple low voltage PIN photodiodes as light detectors. Another considerable advantage is obtained by using a temperature stabilized, single mode diode laser as the light source of the polarimeter. The theoretical measuring accuracy is increased because of the extremely small band width, and the wavelength stability of the temperature stabilized laser. Laser transmission is superior to lamp transmission when measuring dark solutions.

All crucial components of the device are made of light weight materials of long lifetime. Small sizes allow for compact and durable design of the polarimeter. The polarimeter needs only low voltage power supplies to be operated: the energy comsumption is roughly 20 W. Low power consumption, small size and low weight allows designing for portable devices for field use. The optical layout of the polarimeter is shown in Figure 1. In the optical design, care has been taken to minimize interference effects of the coherent light source on all optical surfaces.

Light source

The light source of the polarimeter is a temperature stabilized single mode diode laser, operating at TEM00 single mode, and guaranteeing maximum absolute wavelength stability. Laser light obtained in this manner is coherent, polarized and highly monochromatic. To improve the polarization properties of the light beam an additional polarizing element is used to improve the crosspolarization ratio to better than 10,000 : 1, even at 780 nm. The band width of the laser is 0.02 nm and the absolute wavelength stability is 0.01 nm.

Sample measurement

The sample located in the sample cell and the measuring beam is directed through the sample solution. The beam is collimated by using a lens, and is then polarized parallel to the natural laser polarization further by using an additional polarizer. The beam is then split into two components by an angled beam splitter, which leaves some 4% of the light intensity for the reflected and about 96% of the light intensity for the transmitted beam. The intensity of the transmitted beam is adjusted to the proper intensity level of the experiment by using a neutral density filter, depending on the darkness of the solution used.

By using the laser light to measure the optical rotation of dark solutions, relatively long path lengths, up to 5 cm, may be used. This is an significant improvement as compared to the conventional polarimeters, where path lengths of some 3 mm in the visible spectral region may be used. We estimate that about 20 fold absolute measuring accuracy is gained for dark solutions. This improvement is significant for control of the sugar contents of dark molasses solutions at process sites.

Use of coherent light sources in the study of solutions with small suspended particles results in Mie scattering of light. However, the coherence is lost within the first few micrometers in the cell. Measurements where incoherent light is used experience same scattering. Heating effects (5 mW of CW power) produced by the laser beam in the sample cell are of the order of 0.01 degrees C.

The interest in using NIR-wavelengths has its origin in the enviromental harm caused by lead acetate clarification process widely used in measuring sugar contents of molases and other dark solutions (2).

Rotation speed

In the polarimeter described here (4) the rotation speed of the analyzer is about 50 Hz. Absolute rotation speed is not important but it is advantageous to have the rotation speed constant. This speed allows fast enough rise times of the oscillating signals at the photodiodes to be used for triggering purposes. The signals at both photodiodes are known to obey Malus' periodical law. During one full turn of the analyzer both beams come to cross-polarization position two times giving the effective measuring frequency of about 100 Hz. A crystal oscillator and pulse counting electronics are used to measure the time delay (tx, shift) between the cross-polarization positions of the measuring and the reference beams respectively. The time duration of each turn (td, period) is measured simultaneously from the reference beam by using a second channel of the pulse counting electronics. The absolute angle of rotation is then given by 360° * (tx/td).

Photodiode measurement

Periodical signals are observed at the photodiodes. The measuring signal is automatically amplified (up to a factor of 20) to keep measuring and reference signals at the same intensity levels. This arrangement compensates for possible transmission changes taking place in the sample cell during the measuring process. The polarimeter compares the time delay between the measuring and the reference signals to the time duration for each full turn of the analyzer, giving an internal calibration for each measurement.

Signal averaging is then used to improve the raw data and statistical error analysis is carried out, for further criteria of the quality of the data.

For triggering pulse counting electronics constant fraction discrimination (CDF) techniques are used. The triggering pulse from the reference signal starts two independent counting channels: the stop signals from the delayed measuring signal and the stop signal from the next reference pulse will give the counts for calculating the optical rotation angle. The theoretical resolution, without signal averaging is 0.0018 degrees. Each measurement gives absolute angle of rotation.

To complete one measuring cycle requires a 20 millisecond time period. This comprises a 10 ms measuring time and a 10 ms counter reading time. Using signal averaging for up to 10 seconds will improve the original measuring accuracy roughly by a factor of 20. Standard statistics is calculated at later phases of data acquisition allowing for on-line and long term control of quality of the measured data.

We have made some preliminary measurements on a series of solutions of sugars i. e. sucrose, glucose, invert sugar and fructose of known concentrations. We simultaneously measured the optical rotations of these solutions by a conventional polarimeter and by our laser polarimeter. In all cases, statistics are in favour of the laser polarimeter. For sucrose and for fructose solutions an improvement of measuring accuracy by a factor of ten was obtained. Figure 2 shows the results of the above mentioned solutions, when the laser polarimeter was used.

CONCLUSIONS

We have designed a new laser polarimeter that allows for accurate and very fast measurement of the angle of rotation of optically active solutions. There is no need for quartz calibration of the instrument if the operating wavelength can be assumed to be constant. The measuring range is 180 degrees with theoretical, (and close to practical) measuring accuracy of 0.0018 degrees. The arrangement procures absolute rotation angles for each measurement; measuring times as short as 20 ms may be used. Errors of measurement have their origins in electronic and mechanical noise of the instrument. Both 650 nm (for bright solutions) and 780 nm (for dark solutions), single mode, diode laser light sources may be used with very long lifetimes (typically 20,000 hours). The measurement is totally independent of magnetic fields. User friendly software on an IBM combatible computer will control and record continuos or single measurements.

ACKNOWLEDGEMENTS

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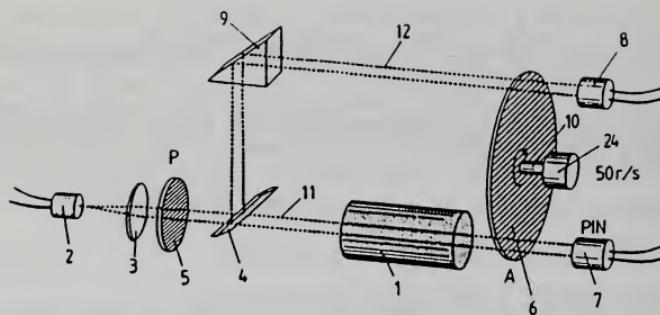
Figure 1. The optical layout of the LP650/780 polarimeter
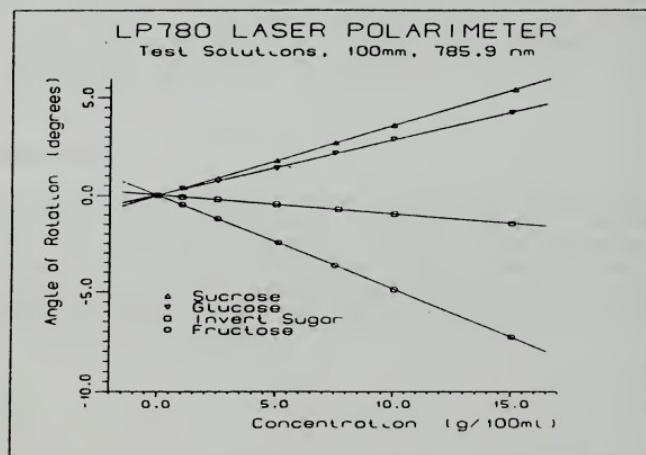
Figure 2. Results for sucrose, glucose, invert sugar and fructose study by using the LP650/780 polarimeter.

DISCUSSION

Question: On the temperature correction, on the effect of temperature on the rotary dispersion: the correction is in two parts: one part on the quartz, and another on the optical rotation of sucrose solution. How is the correction for sucrose solution made?

Korppi-Tommola: We did not use quartz. The measurements reported here were made at room temperature. We control the temperature of the cell continuously, through a signal to the computer. We intend to use the ICUMSA equation to do the correction.

Question: Because of the high intensity of the laser beam, if the intensity is adjusted to a raw sugar of color 3000 ICU, then when a raw sugar of 8000 ICU is measured, it cannot be read. However, if the laser is adjusted to read a solution of sugar of 8000 ICU, then on the less colored sample of 3000 ICU, the laser beam will burn the photo multiplier. The output of the photo multiplier must be modified to the intensity of the laser beam - that's another problem.

Korppi-Tommola: This is not a problem with our approach because we are using photo diodes. We don't have a photo multiplier at all. Photo diodes are more convenient, with low voltages, and a wide response range. If that is not enough, neutral density filters will be used, and electronics will take care of necessary amplification. The power consumption for the whole system is of the order of 40-50 watts - you could put a battery in it and transport it on site to field or factory.

Question: All your test work was done on clear sucrose or glucose solutions. Have you done any testing on colored sugar solutions?

Korppi-Tommola: Yes, we have. The electronics can compensate for colour fluctuations automatically up to a factor of 20. This is accurate within less than 0.05° rotation. Local heating of the laser beam in the sample is negligible.

Question: A simple question of general interest: what is the cost of the instrument?

Korppi-Tommola: As you saw, there are no expensive parts in the polarimeter, except the know-how in the electronics. The polarizer and diodes are fairly cheap. The price will be very competitive.

APPLICATION OF A BIOSENSOR BASED INSTRUMENT FOR THE MONITORING
OF SUCROSE LOSS IN REFINERY WASTE WATER

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ABSTRACT

The evaluation and eventual application of the commercially
available YSI model 2700 Select Biochemistry Analyzer, utilizing a
biosensor, for the monitoring of sucrose loss in refinery waste
water is described in detail.

Emphasis is placed on detection limits, quantitation, loss
estimates and a computerized data communication system that
provides refinery staff with real time information.

INTRODUCTION

In the ongoing operations of a cane sugar refinery, there are
inevitably numerous opportunities which, left unchecked, could lead
to significant sucrose losses.

One of the problems most frequently addressed is loss of sucrose in
refinery waste water. The loss of sugar in what would apparently
appear to be insignificant amounts, parts per million, distributed
through large volumes of water (millions of gallons per day), can
translate into a considerable monetary loss. Sugar can also be a
contributing source to BOD in refinery waste water. Therefore the
monitoring and control of these losses have a direct effect on the
refinery's efforts to reduce the overall amount of pollutants in
their effluent.

With regard to these facts, a number of technical papers can be
found over the years (7,3,4), describing systems, chemistries, and
methodologies for the continuous monitoring of sugar losses in
waste water generated from evaporator and pan condenser operation.
The majority of this work involved the use of a flow injection
Autoanalyzer type system, which was the benchmark of the Technicon
Controls Inc. company for years.

The chemistry applied in these systems is for the most part, based
on the original or modified Hoffman and Wood reaction (1). In this
reaction invert sugars from the acid hydrolysis of sucrose are
determined by the reduction of alkali potassium ferricyanide
(yellow) to colorless alkali potassium ferrocyanide. The reduction

in color (transmitted light) is continually recorded, and directly correlated to the amount of sucrose present.

Other systems for continuous sugar detection reported include: 1) a colorimetric measurement based on the reaction of sucrose with thymol in dilute sulfuric acid, which produces a color from light amber to deep red depending on the sucrose concentration (6), and 2) a system based on classical polarimetry (5).

Over the years our refineries have had experience in implementing almost all of the above systems in one form or another. However, for reasons of cost of replacement, frequency of maintenance, sensitivity, handling and storage of corrosive and toxic chemicals and overall reliability, there was definitely significant room for improvement. Upon our initial introduction and evaluation of the YSI 2700 Select Biochemistry Analyzer, manufactured by Yellow Springs Instrument Co., Inc., it appeared that this instrument could be a viable alternative to the present systems and an answer to some of the shortfalls that are associated with them.

INSTRUMENT THEORY AND APPLICATION

Theory

Selectivity is of paramount importance in the conducting of analytical measurements and is generally achieved by means of selective chemical reactions. Enormously selective and versatile reagents occur naturally in the form of enzymes, antibodies and receptors. Biotechnology has exploited the use of these biological components in analytical systems by the development of immobilized enzymes and immunocomponents which may be re-used (10).

Biosensor technology represents probably the most successful application of this work in the food industry to date. Biosensors are devices which use immobilized biomolecules combined with detectors to respond to specific interactions with environmental chemicals.

Basically there are two types of biosensor differing in the mode of signal generation. Direct bioaffinity sensors utilize a binding event to detect substance. The binding of the incoming analyte to the complementary immobilized bioligand results in a change in the conformation of the biomolecule, and/or physical changes in the immobilization medium such as changes in charge, thickness, temperature, or optical parameters (color or fluorescence). The second basic sensor type is the enzymatic/metabolic biosensor. Here, recognition of the substrate by the immobilized receiver (enzyme) is followed immediately by chemical conversion to the corresponding product, which is detected (2,10).

The YSI 2700 Select Biochemistry Analyzer makes use of the enzymatic/metabolic type of biosensor for sucrose analysis. The YSI sucrose membrane employed in their biosensor consists of three layers with the immobilized enzymes in the middle layer. The outermost layer is constructed of polycarbonate and the inner (against the electrode) is made of cellulose acetate. Three enzymes are employed in the sucrose chemistry: invertase, mutarotase and glucose oxidase. The YSI sensor is shown in Figure 1 (11).

The sucrose in an aqueous environment is broken down by invertase to α-D-glucose and fructose. The α-D-glucose is then converted to β-D-glucose by the action of the enzyme mutarotase. Glucose oxidase will react with the β-D-glucose and available molecular oxygen to form hydrogen peroxide and D-glucono δ-lactone. The electrode measures the amount of hydrogen peroxide produced.

The mechanism for this measurement is as follows; at the enzyme probe there is a direct electrochemical determination of the hydrogen peroxide. A material similar in characteristics to the enzyme membrane is at the probe surface. This membrane contains immobilized nonenzymatic protein to produce diffusion properties similar to the enzyme membrane. The hydrogen peroxide at the platinum anode is oxidized producing two hydrogen ions, molecular oxygen and two free electrons. A dynamic equilibrium is achieved when the rate of hydrogen peroxide production and the rate at which hydrogen peroxide leaves the immobilized enzyme layer are equivalent, and is indicated by a steady state response. The electron flow is linearly proportioned to the steady state hydrogen peroxide concentration and, therefore, to the concentration of the substrate which in this case is sucrose.

Application

The immobilized enzyme technology employed by the YSI 2700 has been in use successfully, predominantly in the clinical field since 1975. However, its use and application in the sugar industry has been little to none.

A recent paper presented at the meeting of the American Society of Sugar Beet Technologists in February 1991 compared a YSI enzymatic analyzer to other methods of sugar determination, namely: high pressure liquid chromatography, ion chromatography, and polarimetery, for accuracy and precision. The findings seemed to indicate that the enzymatic technology lacked the accuracy of chromatography; however, it was faster and sufficiently accurate for factory control (9).

It was these two latter points: speed, and accuracy sufficient for factory or refinery control, as well as the recent availability of optional automated sampling station, that prompted us upon our

initial examination to consider the YSI 2700 as a viable alternative for monitoring sucrose loss in our refinery waste water. It should be noted, that this is not the first use of this technology for this particular application. An Enzymax Analyzer manufactured by Leeds & Northrop was evaluated in a similar application (8). The primary difference between equipment was in the method of immobilization of the enzymes. The Enzymax utilizes a column of beads as a support for the enzymes while as described previously the YSI employs membrane sensor technology.

The direct application of the YSI 2700 as a monitor for sugar in our refinery wastewater entailed a three step program: 1) the initial laboratory evaluation to determine accuracy, reproducibility, etc., 2) a trial plant in-line evaluation and finally 3) direct implementation on an everyday basis.

Results of the laboratory application, which entailed the verification of the detection limits of the instrument, and reproducibility were encouraging. Data displayed in Table 1 illustrate the results obtained for recovery of sucrose in spiked samples of river water. The river water chosen as the matrix for the sample preparation was verified to be sucrose free by ion chromatography. Repeated injections of sucrose concentrations in the area of 0 to 100 ppm sucrose showed only minimal variability. The amount of deviation observed in this experiment did not effect our potential application.

Next the laboratory efforts were directed at optimizing the YSI 2700's operating parameters in the target area of 50 ppm sucrose. This was the level at which the currently installed refinery thymol type detector is set to alarm and notify staff of a potential sugar loss. After numerous trials, an operating standard of 2000 ppm sucrose and an injection volume of 65 microliters were chosen. Listed in Table 2 are the results for 21 repetitive runs of a river water sample spiked with 50 ppm sucrose. It should be noted here that because of better than expected performance of the YSI 2700 when actually placed on line, the standard being employed has been changed to 2500 ppm and the alarm value reduced from 50 to 25 ppm.

At this point it was decided to move to the next stage of the evaluation, namely the placement of the instrument in the refinery for a significant time interval. The instrument was placed on line in parallel with the refinery's currently installed thymol-sulfuric acid detector and operated over a period of three weeks. The only times the YSI instrument was not operating coincided with shut down of refinery operations. No pre-treatment of the water at the sampling point was employed. The instrument was initially programmed to sample every fifteen minutes due to the buffer reservoir capacity (see Figure 2) (11). Appropriate modifications were made and the instrument reset to sample every three minutes.

Over the three week period the average life expectancy of the immobilized enzyme membrane averaged four to five days. Only one instance of line blockage was experienced in the beginning of the third week of the evaluation. The blockage, apparently some sort of scum, appeared to be microbiological in nature. The blockage was cleared easily. To eliminate this maintenance for long term operations, it was decided that some kind of strainer or filter would have to be employed.

As far as the YSI 2700's actual performance, we found it to provide significantly more information than our current detector, responding with quantitative data during periods when our conventional detector appeared to be unresponsive.

Next, the final step of the application, the direct implementation of the YSI 2700 on a daily basis as a monitor of the river effluent from our refinery was initiated. The operating range of the YSI 2700 is from fifteen to thirtyfive degrees centigrade. A determination was made that the environment chosen for the instrument's placement would be less than optimum for any long range operation. The area's temperatures can range from five to forty degrees centigrade with accompanying humidity levels which could reach 100%.

Because of these facts, the need for a controlled climate was agreed on. An environmental enclosure was purchased and assembled. The enclosure or room was equipped with lighting, as well as provisions for heating and air conditioning.

The sampling point for the waste water effluent was the water tunnel where all the possible river return streams combine. The river water is pumped from the water tunnel into a weir box. A sample is drawn from the weir box via a masterflex pump through a fine stainless steel filter into a U-tube. At a pre-programed measurement time interval the YSI 2700, fitted with an external sampling station, enters a purge cycle filling the sampling lines and teflon block sample station with representative waste water. Next the YSI robotic type sipper tube sampler (see Figure 2) (11) extracts a predetermined volume of the water for analysis. Once the analysis is completed the result is stored in memory.

Although the YSI 2700 is a microprocessor based instrument, its capacity for storage of data is limited. In order to increase the storage capability, and make use of the results on a real time basis, a communications and data handling system was devised.

The YSI 2700 is equipped with a RS232 output. This output was connected with a shielded wire to a 286 AT computer located approximately three hundred feet away in the refinery control room. Hardware installations of both a computer relay board and a Metrabyte PDISO-8 board were also required. Software assistance was

provided by YSI in the form of a program written in C language. This program accumulates data from the YSI 2770 and places it in a data file. The program further monitors the values being transmitted for refinery control. A maximum limit is set up in terms of sucrose concentration by a menu selection. If the limit is exceeded, the computer flashes the message "HIGH LIMIT". Additionally, the computer will actuate via relays on the PDISO-8 board three strategically placed alarms.

The placements of the alarms is as follows: 1) at the evaporator station, 2) on the centrifugal floor outside the main control room, and 3) on the pan floor. When a sucrose loss has occurred all three alarms are triggered. This serves to notify everyone, from the operator to the shift supervisor, that a sucrose loss is occurring. Each area responds by checking all instruments and visually inspecting their respective areas to try and determine where and how the loss is occurring. Figure 3 shows a schematic diagram detailing the installed alarm system.

RESULTS AND DISCUSSION

The completion of our evaluation led to the subsequent installation of the YSI 2700 as a continuous monitor for sucrose content in our refinery waste water. It was soon found that data generated from its use provided significantly more information about sucrose loss, and our potential for loss, than did our previous thymol detector. Although the thymol detector provided our staff with relatively consistent monitoring and responded to high level losses, it did little in the way of actually estimating the extent of the loss. The YSI 2700, however, provides quantitative results which when weighed against time and effluent flow can be translated into approximate loss figures.

Examples of the type of data obtained with the YSI 2700 can be seen by viewing the graphs depicted in Figures 4, 5, and 6. The graph in Figure 4, shows data collected by the YSI 2700 during one day (DAY "A") of our refinery operation. Notice that during no time was the preset alarm limit of 25 ppm exceeded. However, there are three instances where the instrument detected a measurable sucrose concentration in our outgoing waste water. The peak concentrations for these instances ranged from approximately 6 to 8 ppm to a high of 23 ppm.

The graph in Figure 5, for DAY "B", shows little overall sucrose loss except for one instance at 40.1 ppm, between 9:44 AM and 10:00 AM.

Unlike the two previous graphs illustrating short and sporadic periods of sucrose detection in the refinery effluent water, YSI data in Figure 6 show a significantly lengthy interval (approxi-

mately fourteen hours) of measurable sucrose loss. Even though the measured levels exceeded our 25 ppm limit during a couple of intermittent spikes, the average level of loss over the fourteen hours was only approximately 6 ppm.

Figure 7, shows an example of use of the YSI 2700 data not only to monitor, but also quantitate the amount of sucrose lost in waste water. Note that the recorded measurement interval for this data extended from 9:46 PM to 10:54 PM or approximately 68 minutes.

Integrating the area under the curve in Figure 7 over the time interval from 9:46 PM to 10:54 PM, and applying the appropriate calculations, an average concentration of sucrose in the waste water effluent can be obtained for this period. Knowing the actual or employing a nominal value for the rate of the return flow of the refinery waste water, a figure for the total amount of sucrose lost for the sixtyeight minute interval can also be calculated.

Finally, the data Figure 8 in many ways epitomize the need for the for the monitoring of refinery waste water for sucrose. Referring to the graph in Figure 8 of the YSI cumulative data for the day ("Day D") on which the data depicted in Figure 7 was recorded, a spike appearing at approximately 6:00 PM can be observed. This spike according to detailed data lasted for a period of approximately 18 minutes from 6:10 PM to 6:28 PM. However, what is more significant is that this may have been a precursor or indication of the larger problem which eventually occurred between 9:00 and 11:00 PM.

Although this particular data was collected during the YSI 2700's initial evaluation, prior to its permanent installation including communication and alarm hook up, it still demonstrates some of the instrument's obvious advantages over most of the conventional sucrose detectors. However, in spite of its advantages, the YSI 2700 was found to have its own share of problems.

Currently the YSI 2700 has been on line monitoring our refinery's river water effluent for approximately nine months. The major problem to date was experienced during the first four months of installation and dealt with the quality of the immobilized enzyme membrane. During this period, the sucrose membranes received from YSI Incorporated exhibited a very poor response. Probe currents of 10 to 15 nanoamps were generated by new membranes for our standard calibration solution and injection volume. Additionally, extended intervals for the instrument's calibrations were also noted. These observations which were discussed in detail with YSI Incorporated led to the identification and eventual solution to the problem.

Apparently YSI Inc. had been experiencing problems dealing specifically with the mutarotase enzyme and were experimenting with

more efficient immobilization techniques. Membranes obtained
during the following two months after the problematic initial
interval were improved, exhibiting working life spans of approxi-
mately one week. Since this time the sucrose membranes produced
have been of even better quality. Probe currents of 35 to 50
nanoamps have been obtained for a 65 microliter injection of our
calibration standard containing 2500 ppm sucrose. Newly installed
membranes have been found to remain in operation for intervals of
up to four weeks without failure. It appears that problems
surrounding the quality of the immobilized enzyme membrane have
been effectively addressed and are no longer an issue. Illustrated
in Figures 9 and 10 are current versus time graphs which, when
compared, show the difference in responses between membranes of
poor and acceptable quality.

Other minor problems of note were experienced with some of the
mechanical and electrical components of the instrument, specifical-
ly the pumps, robotic arm mechanism and power supply. Failure of
some type dealing with each of these components has occurred. All
the reasons for failure in each case have yet to be determined.
Therefore, it is our opinion, that appropriate spare parts for
these components need to be stocked on site in order to avoid
significant downtime.

Finally, in our present installation of the YSI 2700 as a sucrose
detector, the instrument is connected to an average quality spike
inhibitor. Because of the prevailing variability in electrical
supply associated with a large industrial operation such as a cane
sugar refinery, we are currently considering the use of an on line
uninterruptable power supply.

SUMMARY

This paper has detailed the application and implementation of a
Yellow Springs Instrument's 2700 Select Biochemistry Analyzer as a
sucrose loss detector for refinery waste water.

The benefits and advantages, as well as problems associated with
its use, are discussed at length in the light of our experience and
actual performance data.

Overall the benefits received by employing the YSI 2700 in terms
of: 1) the amount of data produced, 2) the increase in accuracy, 3)
the relatively low cost, and 4) the reduction in the inventory and
use of dangerous chemicals were found to outweigh any obtained with
our past sucrose loss detection system.

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Table 1. - Recovery and detection of sucrose in spiked river
water samples.

Sucrose Spike Concentration, ppm	*YSI 2700 Result, ppm	% Recovery
0	0	----
10	7.1	71.0
20	18.6	93.0
40	39.7	99.3
60	59.5	99.2
80	78.6	98.3
100	93.5	93.5
0	1.5	----
10	11.5	115.0
20	18.3	91.5
40	38.4	96.0
60	60.5	100.8
80	79.5	99.4
100	99.8	99.8
0	0.8	----
10	8.0	80.0
20	17.3	86.5
40	40.1	100.3
0	0	----
10	10.3	103.0
0	0	----
10	8.9	89.0
Average % Recovery		95.0

* The YSI 2700 was re-calibrated after ever five samples.

Table 2. - Variability of YSI 2700 for multiple
determinations of a 50 ppm sucrose spiked sample.

Determination #	*Result Sucrose, ppm	
1	50.9	
2	52.9	
3	48.0	
4	49.6	
5	46.5	
6	49.5	
7	47.6	Standard Deviation
8	50.6	
9	51.6	2.67
10	43.3	
11	49.7	Average
12	50.7	
13	49.8	48.41
14	51.1	
15	44.8	Range
16	45.0	
17	45.8	43.3 - 52.9
18	49.7	
19	47.6	
20	43.4	
21	48.5	

* The YSI 2700 was re-calibrated after every five
determinations.
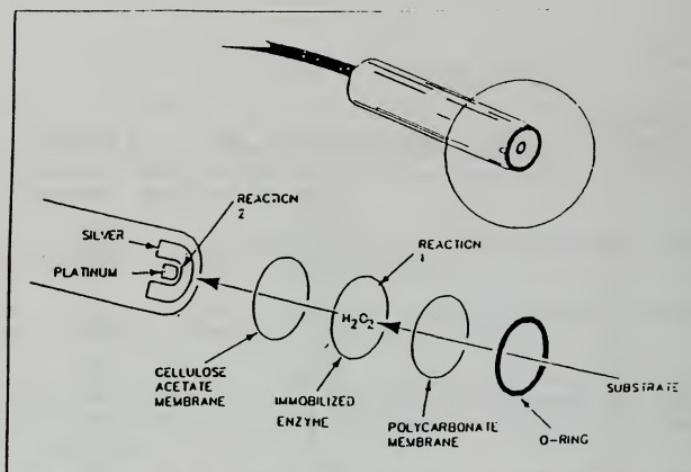
FIGURE 1. - YSI SENSOR PROBE AND ENZYME MEMBRANE
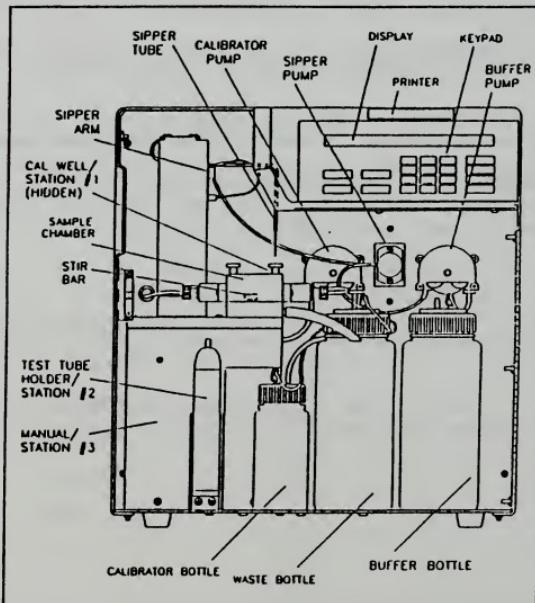
FIGURE 2. - YSI 2700 MAJOR COMPONENTS

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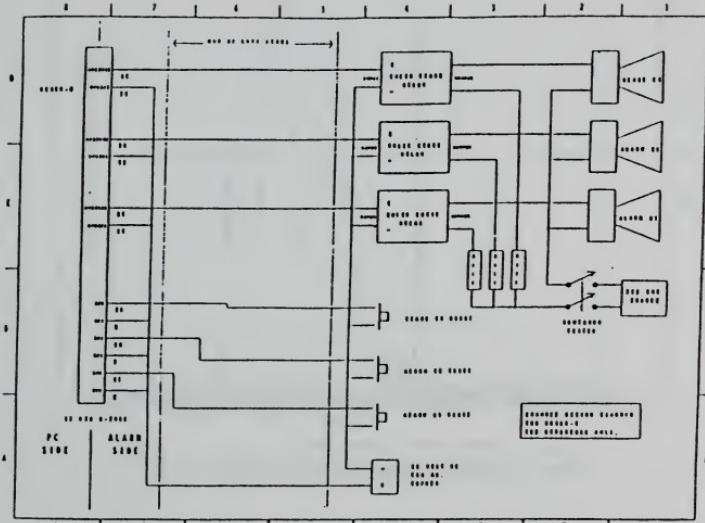
FIGURE 3. - SCHEMATIC DIAGRAM OF PRESENTLY INSTALLED ALARM SYSTEM
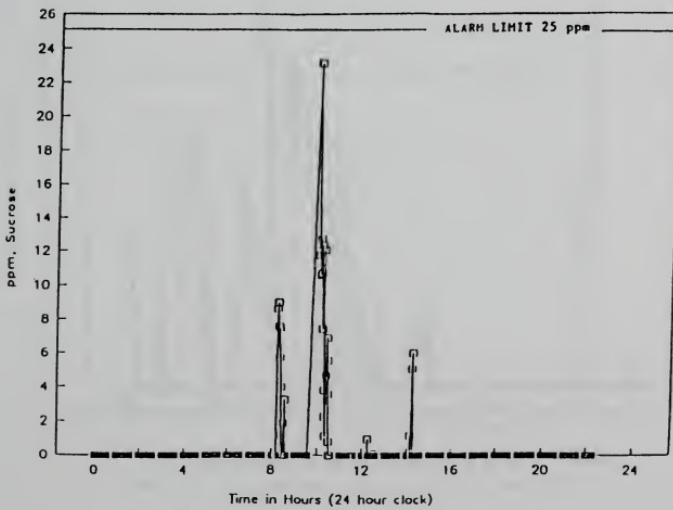
FIGURE 4. - WASTE WATER MONITORING BY YSI 2700 - "DAY A"
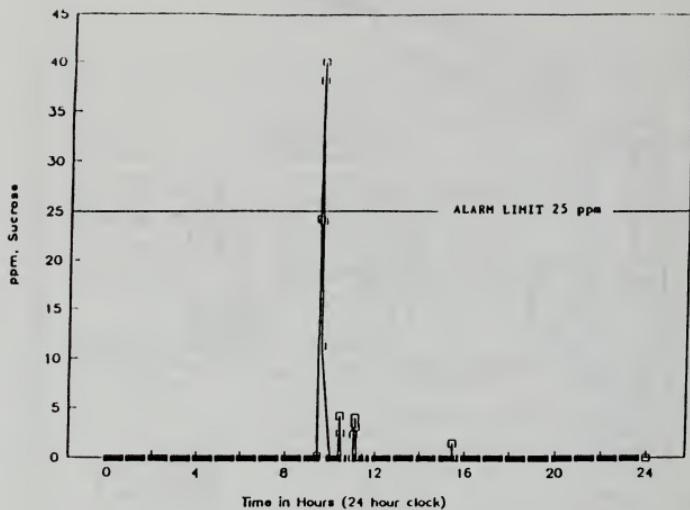
FIGURE 5. - WASTE WATER MONITORING BY YSI 2700 - "DAY B"
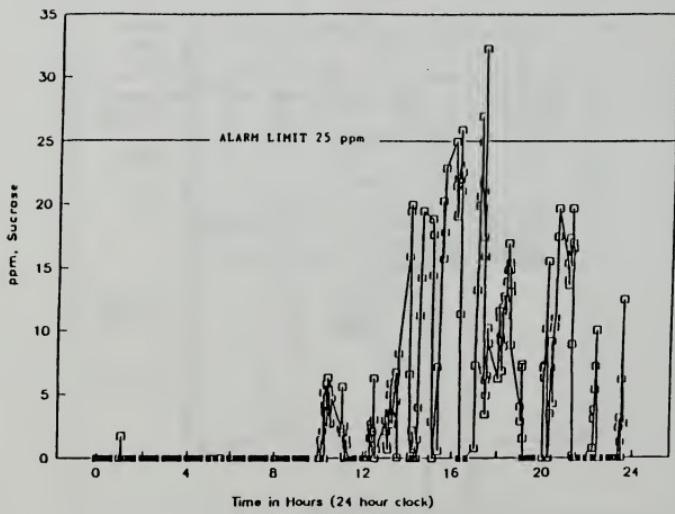
FIGURE 6. - WASTE WATER MONITORING BY YSI 2700 - "DAY C"
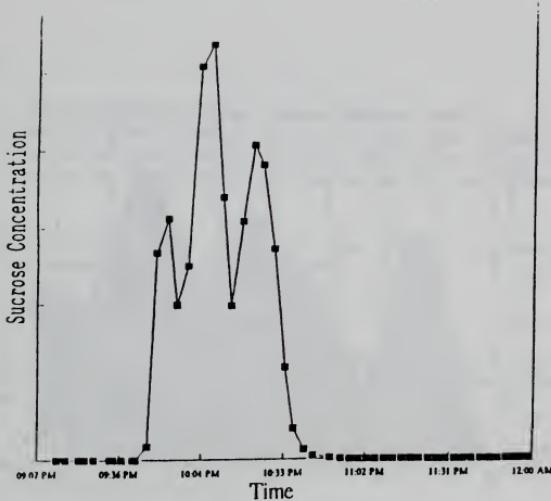
FIGURE 7. - THE APPLICATION OF YSI 2700 DATA TO THE
QUANTITATION OF SUCROSE LOSSES
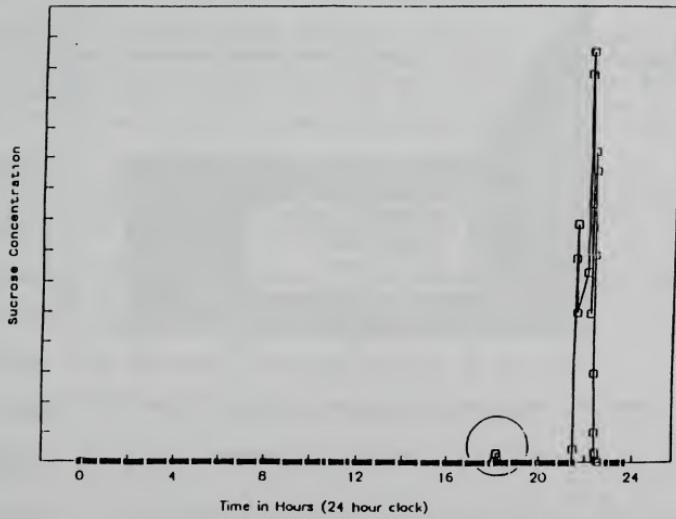
FIGURE 8. - YSI 2700 CUMULATIVE DATA RECORDED FOR "DAY D"
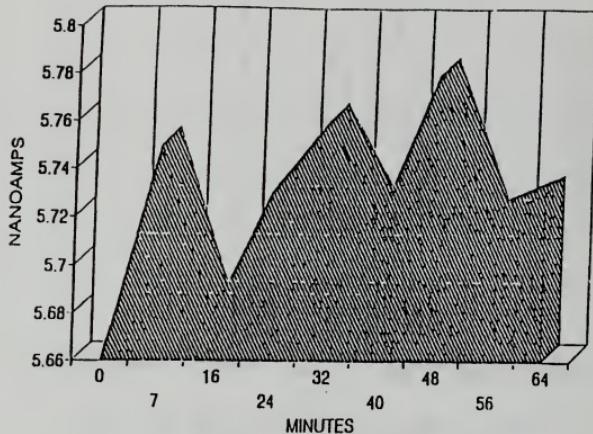
FIGURE 9. - GRAPH ILLUSTRATING RESPONSE OF POOR SUCROSE MEMBRANE

SUCROSE MEMBRANE
LOT 92E47001B
EXP. DATE 11/92
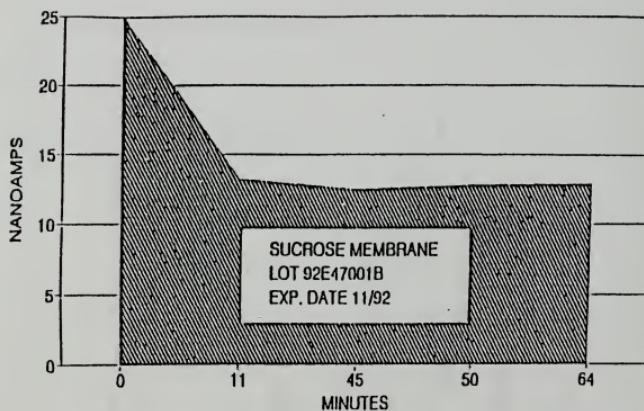
FIGURE 10. - GRAPH ILLUSTRATING RESPONSE OF ACCEPTABLE SUCROSE MEMBRANE

DISCUSSION

Question: The response from the glucose oxidase membrane is much greater from glucose itself than it is from sucrose that's been inverted. In a waste stream you're likely to have some invert. I wonder if you're attempted to compensate for any glucose that could be present?

Angone: Chromatographic analysis has shown us that the sugars in our waste streams are more than 99% sucrose.

Question: Are you using only one probe?

Angone: It's a two probe system. You could do both sucrose and glucose, but we just test for sucrose using alternate probes so the machine is never down.

Question: You talked about the environment for the instrument at 35°C. What is the maximum temperature of the sample that's acceptable to the instrument?

Angone: That's not a factor, because we sample 65 μl into a large quantity of buffer, so that lowers the temperature of a sample that might be at 100°C. The ambient temperature affects the buffer temperature which is the determining factor.

Question: In the spiked sucrose solutions used for calibration, the recovery values were non-linear. For the purpose of process control to initiate alarms, this is probably not an issue. Do you just accept that error? How do you calibrate the instrument to operate in the range of the values you showed?

Angone: A good feature of this instrument is that at any one calibration point (we used 25 mg/kg) it is linear throughout the range we are interested in. When set at 25 mg/kg, the samples showed 43-53 mg/kg with data confirmed by HPLC.

Question: I am interested in the response time for this instrument. How long will it take for a sudden change in sugar content of waste stream to show up?

Angone: The chemistry actually takes 35 seconds.

Question: Is that the actual response time?

Angone: Actually response time has varied from 1 minute to 4 minutes.

Question: A comment: this unit is used on condenser water, where there is not much suspended solids. Another unit will be installed on industrial waste water, which may have 3000 ppm suspended

solids. We'll probably have to put a membrane filter unit in front of the detector to remove the suspended solids.

Question: Two questions: one, what the instrument costs and what it saves you; and two, when an alarm does go off, what is the action taken at the refinery?

Angone: The instrument cost is around $12,000. My project cost over that, because we built a 4'x6' insulated room, equipped with an air conditioner and heater to maintain 25°C ± 5°C.

For response to an alarm: the supervisor, who is already seeing trends on his computer, will check tank levels, and look for broken vacuums; the evaporator station operator will look for high levels in the evaporators on gauges and site glasses. The people on the pan floor will check that they did not break vacuum, or have foaming over, and will check the sight glass. There's a series of responses on the refinery floor. Responsibility for losses has been transferred from the laboratory to the individual process operations, where it belongs - we get a much quicker response.

PARTICLE SIZE MEASUREMENT SYSTEM BY LASER LIGHT DIFFRACTION

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ABSTRACT

Experience on the laser light diffraction method used for process
control is described. The instrument replaces screening in the
determination of particle size of all dry sugar products apart from
the very coarsest. This method gives more rapid and reliable
results than screening.

INTRODUCTION

The sugar refinery at Arlöv normally refines about 120 000 tons of
raw sugar each year. The refined sugar from the first, second and
third classes is mixed together to form a standard sugar, which
later on becomes raw material for production of cubes, pearl sugar,
icing sugar, different kind of granulated brown sugars, sugar
solutions etc. For many of the products the particle size is an
essential characteristic for the quality of the final product. It
is important that the quality control use analytical methods which
are both rapid and reliable, so the production personnel can adjust
and run the factory as effectively as possible.

The particle size for standard sugar is one of the characteristics
checked every hour in order to ensure good quality for final
product. Particle size determination with a set of sieves and a
mechanical shaker is not a rapid method and is not suitable for
sugars having abnormal distribution nor for finely powdered sugars.
By results are available, 10-20 tons of sugar have already passed
from the drier to the silo.

METHODS AND MATERIALS

About two years ago our laboratory bought equipment for particle
determination by means of laser light diffraction. The equipment is
manufactured by the German firm Sympatec (Sympatec GmbH,
Burgstätter Strasse 6, D-3392 Clausthal-Zellerfeld) and carries the
product name HELOS, an abbreviation for HElium neon Laser Optical
diffraction Spectrometer. Today, the system costs about US$ 80,000.

In addition to HELOS there is also a dispersion system called RODOS, which makes it possible to use the laser light diffraction method for dry products and to measure over a range from 1.8 up to 1.5 mm.

The dispersion process with RODOS (Figure 1) begins with the transfer of the mass flow to the injector. First, the material is extracted from the rotary table by suction. Then, as it passes along the injector, the material mixes with transporting air, which accelerates progressively. This results in dilution and initiates the isolation of the particles. The rotary table is used exclusively for feeding fine products. Coarser sugar is fed into the injector through a simple funnel.

The equipment (Figure 1) consists of an optical bench system, short for small particles and long for the coarser ones, a He-Ne laser, an optical beam expansion system, deflection mirrors, collecting lenses with different focal lengths and a detector. Particles in the path of the laser beam cause diffraction patterns measured by the detector. Spherical particles of the same size may be stationary or in motion, suspended in a liquid or gas: the diffraction pattern remains the same. The pattern consists of an extremely bright central point and a number of concentric light and dark rings. These rings are called Fraunhofer Rings, after the man who first described the phenomenon in 1817.

The intensity and mutual spacing of the rings can be correlated to the size of the particles. However, if the particles in question are not of the same size, everything becomes considerably more troublesome. The diffraction pattern becomes diffuse and the calculation required to relate this to the particle size becomes extremely complicated.

In order to read the resulting diffraction pattern, a special and extremely sensitive light detector is placed in the focal plane of the lens; the detector scans the diffraction spectrum at a rate of 240 times per second. The complicated calculation is carried out by a computer connected to the system. This enables us to disregard the advanced mathematics involved and merely to ascertain that the system does work. A particle size analysis takes only 10 seconds.

Data presentation by the HELOS indicates particle sizes in microns (μ) instead of millimetres (mm). Another difference, which perhaps requires more explanation, is that HELOS always indicates the percentage of samples FINER than each level, while the screen method indicates exactly the opposite, the percentage of samples COARSER than each screen. As a result, the x16 of the screening corresponds to the HELOS x84, while x84 consequently corresponds to

x16. After some use of this equipment, the different terminology does not present any problems. Figure 2 shows the HELOS presentation both in tabular form and in a chart. The chart presents both the cumulative curve with particles finer than each level and the histogram on the distribution.

The measurement principle of laser light diffraction is based on the assumption that all particles are spherical in structure and, of course, this is not true of sugar crystals. Our subtances consist of more or less oblong crystals. HELOS measures the longest axis of the crystal and perceives this as the diameter in a spherical particle. In screening, we obtain exactly the opposite result is obtained: a number of crystals will "stand on end" and pass through the screening cloth. When this is the case it can be said that screening measures the shortest axis of the crystal.

CALIBRATION AND COMPARISON WITH STANDARD METHOD

This instrument was to replace screening in the determination of particle size of all dry sugar products, except for the very coarsest, such as pearl sugar. We were fully aware of the fact that analysis results produced using different methods can never be directly compared and that the sources of error vary from method to method.

The idea was not to find any universal relationship between the two methods, but merely to make a simple comparison and attempt to find a working relationship which makes it possible to relate rapidly the methods to one another and establish norms for particle analysis according to the HELOS method. The investigation to quantify the difference and translate the norms has given us a rapid method for product control operating with HELOS.

The first thing we did was to calibrate both our sieves and the HELOS equipment with calibrated glass beads (National Bureau of Standards, Std. reference material 1018a, U.S. Dept. of Commerce).

Figure 3 shows the cumulative size distribution for the NBS standard glass beads. Results from screen analyses (repeated twice) show a small difference from the HELOS results, because it is impossible to use the glass beads more than once without a small loss of weight. We used the beads a third time for the HELOS analysis; because of the small loss of weight, the HELOS result differs slightly from the two screen results. Figure 4 shows the calculated difference before and after calibration, both for the sieves and for the Laser value. The results are acceptable for spherical particles but sugar crystals are not spherical.

Whichever method is used, the sieving or the HELOS method, the manner in which the sugar sample is taken from the process, and how it is handled before particle size analysis, is exactly the same (the sugar sample MUST be representative). It is extremely important to split up the sample in the correct way and then to use all of it. A sample divider (Retsch), divides the sample taken from the batch into 8 equal portions. Figure 5 shows results from these analyses. Figure 6 shows results when a spoon has been used to take out a sample from the bottom and the top of the sample container; considerable differences may be observed.

HELOS gives higher values than screen analyses; with a smaller difference for fine powders than for coarse. The particle analysis of icing sugar was a particular problem so and so was the frost investigation. For icing (powdered) sugars wind screening equipment (Alphine) is normally used. A particle size analysis takes at least half an hour and is both dusty and noisy. The smallest particles detectable are 32 μ. With HELOS analysis takes only 10 seconds and provides information about the very fine particles (1.8-32 μ) which is not obtainable from wind screening. It is not difficult to understand why the HELOS method of analysis has so quickly been adopted for production. The histograms from production of icing sugar with different speeds on the mill in Figures 7 and 8 show it is easy for the production personnel to remember the picture of a good product.

For granulated sugar more extensive investigations have been made. The icing-sugar study revealed that the Laser method was better at detecting particles finer than 200 μ. Particles of under 150 μ were added to a granulated sugar to establish what percentage of these particles could be detected using the Laser and Screen methods. Results are shown in Table 1 and Figure 10. The finer the particles, the greater the difference is when using the screen method. The results of the Laser method are quite acceptable.

RESULTS AND DISCUSSION

In the production control of standard sugar, the medium particle size, the coefficient of variation and percent of particles finer than 150 μ are checked every hour. During the spring, these checks were made using both the Laser and the Screen methods for 150 comparison analyses. For particle medium size, the Laser result must be divided by (1.09x1000) in order to transform it to the same presentation result as the screen result, shown in Figure 11. For the coefficient of variation, the result must also be divided by 1.09 as shown in Figure 12.

Results show that the standard deviation for the medium size particle is +/- 6% and for the coefficient of variation there is a standard deviation of +/- 10%.

In addition to calculated value for medium particle size and coefficient of variation, collaborative tests were carried out at six different laboratories on ten different occasions. These are the results:

The variation from the mean value for the medium particle size, shown in Figure 13, is:

+/- ca 10% for the laboratories using the Screen method and
+/- ca 5% for Arlöv using the Laser method.

The variation from the mean value for the coefficient of variation, shown in Figure 14 is:

+/- ca 9% for the laboratories using the Screen method and
+/- ca 7% for Arlöv using the Laser method.

Use of the Laser method was therefore deemed acceptable. The relationship works well, and with an even smaller deviation than for the laboratories using the screen method.

Different fractions from the sieves in the Screen analysis method show somewhat confusing results, so far, without explanation.

Figure 15 shows the different screen fractions analysed with HELOS. It is quite clear that the coarser the sugar crystals are, the wider is the variation of particle sizes in the HELOS analyses. There seems to be a connection between the top of the curves and the maximum particle size from the screen, as shown in Figure 16. No further investigation has been made of these results since the translation of medium particle size and coefficient of variation is quite adequate for production control.

Another application was made during the latest beet campaign. A suspension cell for particles in liquid was added to the equipment to enable a particle analyses of the "slurry" for the crystallisa-tion pans. When using this wet-dispersion method it is absolutely necessary that the suspension medium is thoroughly saturated with sugar before the sample is added. By circulating the crystals through the laser beam there is no difficulty in analysing the particle sizes.

CONCLUSIONS

The Laser method can replace screening in the determination of particle size for dry sugar products from 1.8 μ up to 1.5 mm. It is possible to get a relationship for x50 (medium particle size) and CV (Coefficient of variation) between the two methods.

For very fine products like icing sugar (powdered sugar) there is no doubt that the Laser method gives better information about the particle size than screening.

Among the advantages of the Laser method are: Speed, reliability, usefulness for powdered sugars, and provision of better data for a better fact foundation in discussion with customers.

Among the disadvantages are the expense, the lack of complete correlation to traditional methods, and the lack of application to coarser products.

REFERENCES

1.   Allen, T. (1990). Particle size measurement. 4 ed., 736 pp.

2.   Röthele, S. and U. Kesten. The Application of Laser Diffraction Technology.

Table 1.   Detection of crystals finer than 150 microns added to a
           sample of granulated sugar.

%	Total		Calculated		Analysed	
Added	Weight	Gram	%	Added	Laser	Screen
0	50.0	1.15	2.3	0.0	0.0	0.0
1	50.5	1.65	3.3	1.0	1.0	0.9
2	51.0	2.15	4.2	1.9	1.6	1.8
3	51.5	2.65	5.1	2.8	2.8	2.6
4	52.0	3.15	6.1	3.8	3.6	3.4
5	52.5	3.65	7.0	4.7	4.3	4.4
6	53.0	4.15	7.8	5.5	5.5	5.0
7	53.5	4.65	8.7	6.4	6.4	5.7

1. RODOS, dry dispersing system
2. Optical bench system
3. He-Ne-Laser with beam expander
4. Deflection mirrors
5. Collecting lenses
6. Detector with autofocus-system
7. Particles in the Laser beam
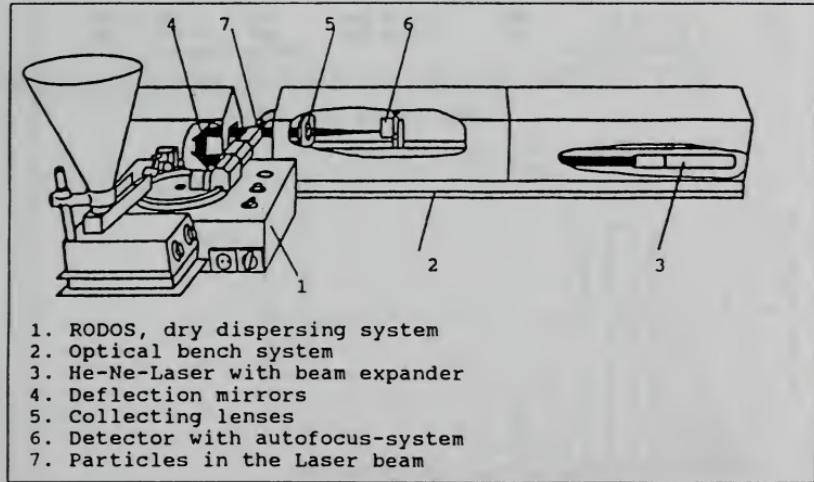
FIGURE 1. Prospective view of HELOS/RODOS system

```
            * * *  ARLÖVS SOCKERBRUK - Laboratoriet  * * *

            S Y M P A T E C   H E L O S  Particle size analysis

Measure method = Torr dispergering (RODOS)              Cascade = nej
Pressure        = 0,5  bar                  Injector depression = max   mbar
Feed rate       = 22   %                    Rotation rate       =         %

Focal length   =  1000 mm          Date              13.07.1992 / 13:34
Measuring time =    15 s           Filename     C:\H_DATA\K5TEST\XX_3.HEL

Operator   = ARS
Sample name = JÄMF. SIKT M/U SLAG

Comments1  =
Comments2  =

   x/mym   Q3(%) I   x/mym    Q3(%) I   x/mym    Q3(%) I   x/mym    Q3(%)
---------------------------------------------------------------------------
           I   31.00   0.23 I  125.00    1.05 I  510.00    43.11
   9.00   0.09 I  37.00   0.25 I  150.00    1.64 I  610.00    58.42
  11.00   0.11 I  43.00   0.28 I  180.00    2.74 I  730.00    74.54
  13.00   0.13 I  50.00   0.31 I  210.00    4.33 I  870.00    88.28
  15.00   0.14 I  60.00   0.36 I  250.00    7.34 I 1030.00    96.91
  18.00   0.16 I  75.00   0.46 I  300.00   12.46 I 1230.00   100.00
  22.00   0.19 I  90.00   0.59 I  360.00   20.21 I 1470.00   100.00
  26.00   0.21 I 105.00   0.75 I  430.00   30.60 I 1750.00   100.00

x10   = 275.98 mym       x50   = 554.98 mym        x90   = 901.84 mym
x5    = 218.87 mym       x16   = 327.39 mym        x84   = 826.37 mym
Sv =  0.0148 m**2/cm**3                    Optical concent.= 4.3 %
```


FIGURE 2. Helos presentation of a particle size analysis

CALIBRATION OF SIEVES AND LASER WITH CALIBRATED GLASS BEADS
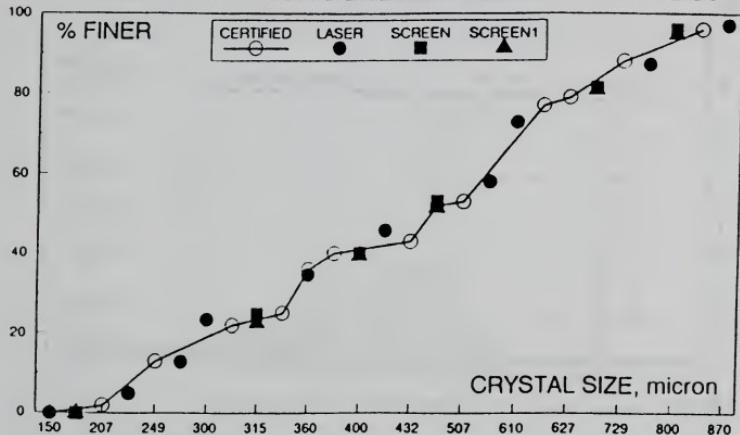
FIGURE 3. Analyses of Glass Beads from the National Bureau of Standards.

% DIFFERENCE BEFORE AND AFTER CALIBRATION
SIEVES AND LASER VALUE
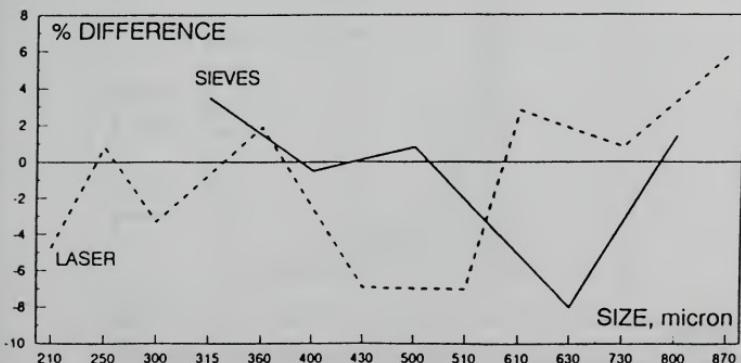
FIGURE 4. % Deviation before and after calibration with Glass Beads.

ANALYSES OF SAMPLES FROM THE SAMPLE DIVIDER
SEVEN EQUAL PORTIONS
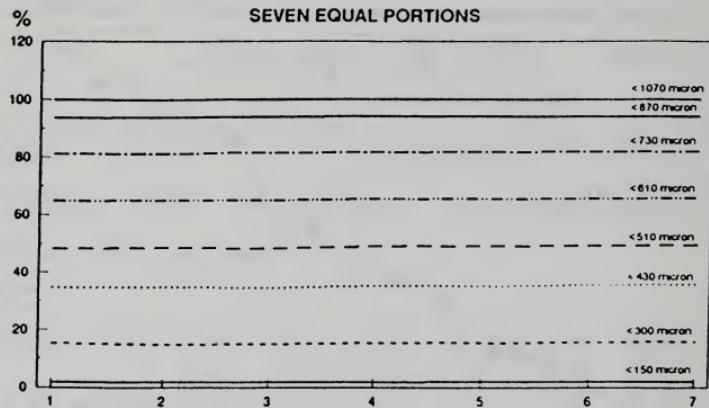
FIGURE 5. Seven equal portions analysed from the Sample Divider.

ANALYSES OF SAMPLES TAKEN WITH A SPOON
FROM THE BOTTOM AND THE TOP OF THE CONTAINER
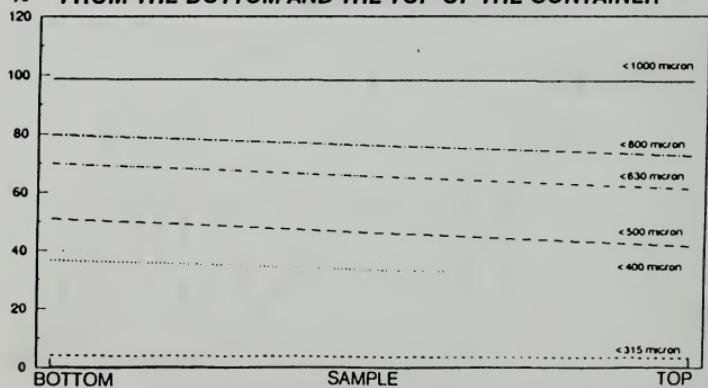
FIGURE 6. Samples analysed from the bottom and the top of the sample container.
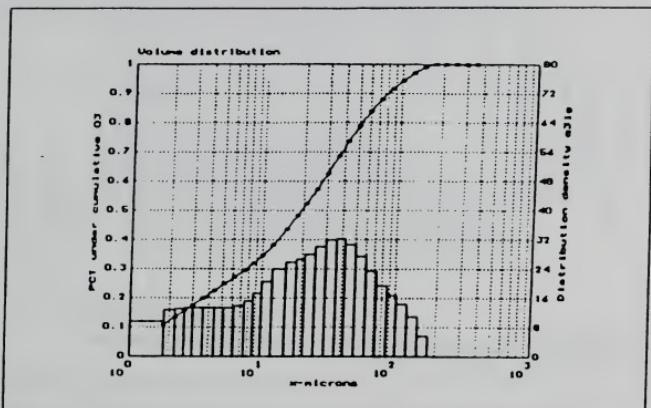
FIGURE 7. Icing sugar manufactured with a higher speed on the mill.
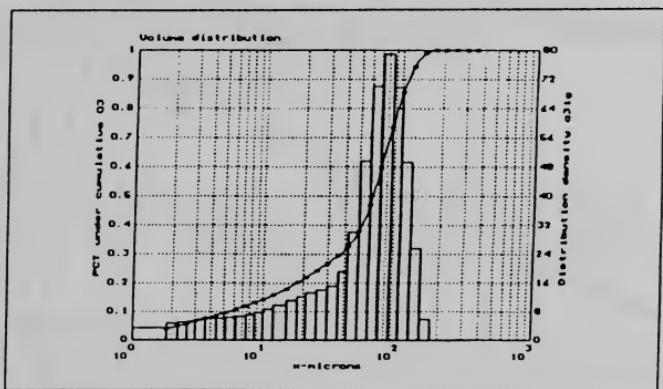
FIGURE 8. Icing sugar maufactured with a slower speed on the mill.
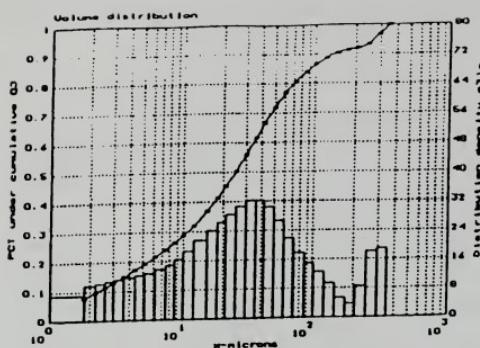
FIGURE 9. An icing-sugar with too many coarse particles.

DETECTION OF CRYSTALS FINER THAN 150 micron
DIFFERENT QUANTITIES ADDED (0·7%) TO A SAMPLE
OF GRANULATED SUGAR
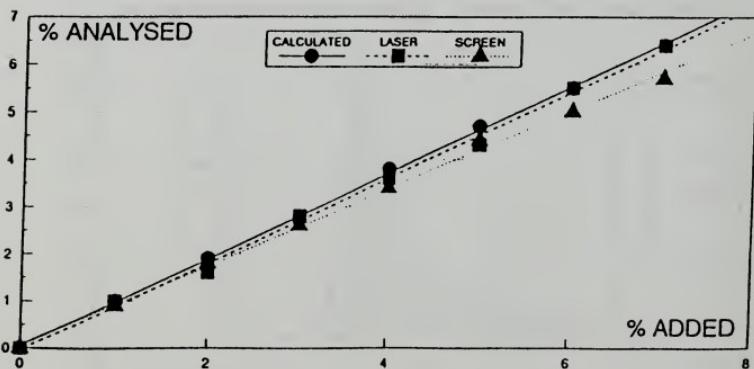
FIGURE 10. Detection of crystals < 150 micron with Laser and Screen method.

MEDIUM PARTICLE SIZE (a50)
COMPARISON BETWEEN SCREEN AND CALCULATED LASER
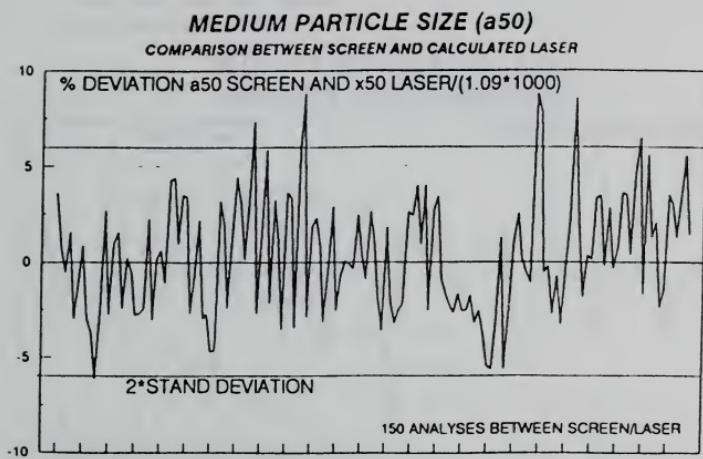
FIGURE 11. Comparison between screen value and calculated Laser value.

COEFFICIENT OF VARIATION (CV)
COMPARISON BETWEEN SCREEN AND CALCULATED LASER
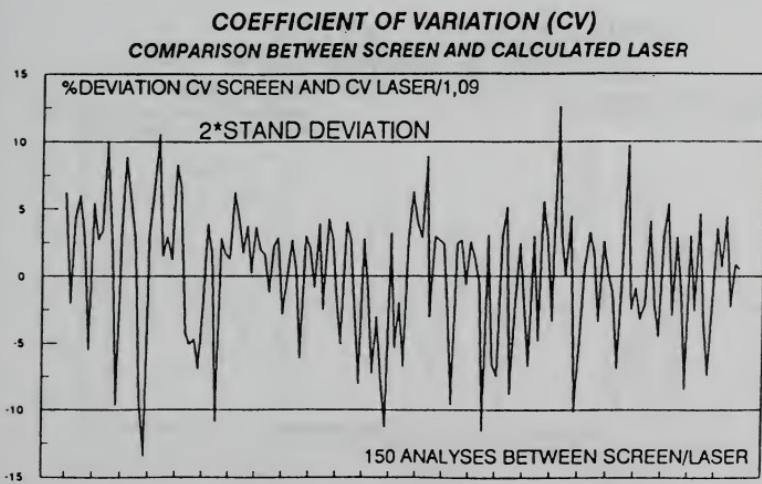
FIGURE 12. Comparison between screen value and calculated Laser value.

COLLABORATIVE TESTING
MEDIUM PARTICLE SIZE (a50)
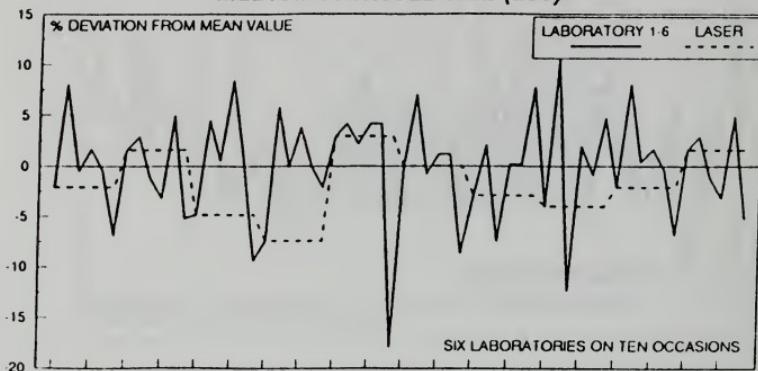
FIGURE 13. Collaborative testing of medium particle size.

COLLABORATIVE TESTING
COEFFICIENT OF VARIATION (CV)
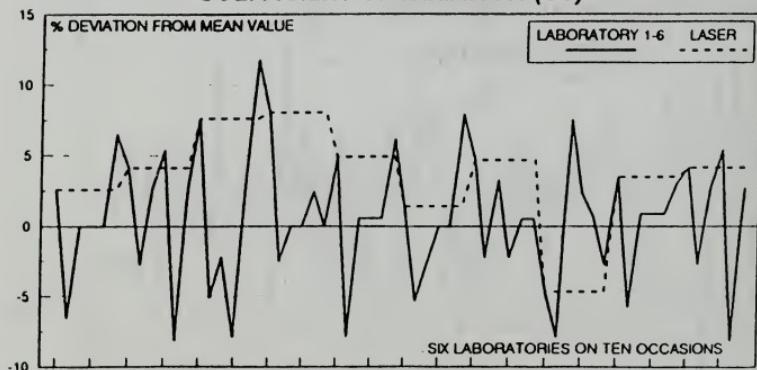
FIGURE 14. Collaborative testing of coefficient of variation.

FIGURE 15. Fractions from the sieves analysed with HELOS.
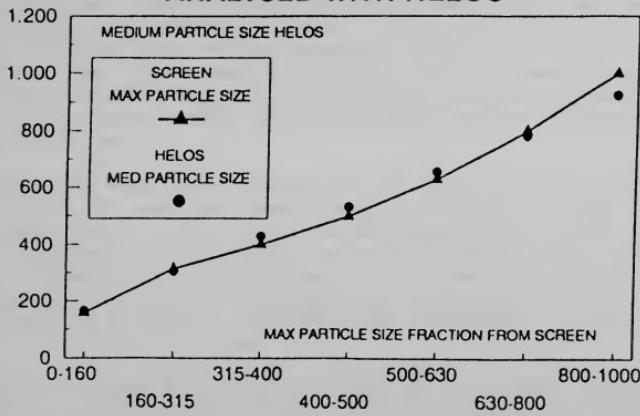
FIGURE 16. Connection between HELOS x50 and the sieves max value.

SPRI

DISCUSSION

<u>Question:</u> In your written paper, you have emphasized the impor- tance of taking the sample from the process stream. Can you comment on how and where you take them?

<u>Olofsson:</u> We take the sample after the drier, directly, while it is falling down. You can't take the sample from a conveyor belt because the crystals have separated then. We have future plans to use the Laser method on line.

<u>Question:</u> What is the type of detector in the instrument: the old ring type or a CCD?

<u>Olofsson:</u> Silitium multielement detector.

<u>Question:</u> Which icing sugar tastes better: the one with Gaussian distribution or the one with Poisson distribution? (This question is facetious)

<u>Chairman:</u> This very relevant analytical development will become more and more important as we improve quality control, and move more towards ISO 9000. We will be forced to provide more data, and the Laser system just described is the sort of tool that will provide the answers we need.

CAN HPIC REPLACE GC AS A VIABLE, ACCURATE, ROUTINE PROCEDURE FOR
DETERMINING CARBOHYDRATES IN SUGAR CANE JUICES AND FACTORY
PRODUCTS?

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Institute, Durban, South Africa

ABSTRACT

Because of inaccuracies in polarimetric and chemical procedures,
gas chromatography (GC) has been used in the South African Sugar
Industry to determine cane quality and factory performance for the
past 13 years. As the GC procedure suffers from a number of
disadvantages, the Sugar Milling Research Institute is busy with an
ongoing investigation into the use of an accurate HPLC technique
for rapid and simple carbohydrate analyses. The advantages and
disadvantages of the different HPLC procedures, including HPIC (ion
chromatography), are discussed. Reasons for the possible adoption
of ion chromatography for the determination of fructose, glucose
and sucrose in juices and molasses products are given.

INTRODUCTION

The South African sugar industry has always believed that good
analytical control is the foundation for optimising/improving sugar
recovery in raw sugar mills. With this in mind, the industry (3)
introduced gas chromatography (GC) in 1979 for the analysis of
sugars in both mixed juice (MJ) and molasses. Schäffler and Smith,
1978, showed that this decision cleared up many of the anomalies
that had previously resulted from the use of empirical analytical
procedures.

Crushing seasons in South Africa are relatively long (40 weeks) and
weekly MJ and molasses samples from at least 16 factories are
dispatched to two central GC laboratories for analysis. It is
estimated that 50,000 MJ/molasses samples will have been processed
by these two laboratories by the end of the 1992 season. Apart
from these routine analyses, GC has been utilised in many trouble-
shooting projects dealing with inversion, entrainment and invert
degradation problems.

Before samples can be analysed by GC, they need to be derivatised
using a two-stage process (9). The sugars are separated on a fused
silica capillary ensuring that any overlap due to co-eluting
impurities is reduced to an absolute minimum. Statistical data
emanating from this programme have shown that the data are highly

reliable and in its 13 year history, deadlines were missed on fewer than 10 occasions. The local sugar industry is obviously extremely satisfied with GC, yet research at the Sugar Milling Research Institute has continued trying to find an HPLC alternative.

There is little doubt that GC separations of sugars are still superior to those obtainable by HPLC. GC does however suffer from a number of disadvantages. In terms of routine analysis, the main drawbacks of GC for sugar analysis are:

(a) <u>Speed</u>. Duplicate samples for each factory go through many analytical procedures including two-step derivatisation. This makes it difficult to prepare and chromatograph samples on the same day. With HPLC, samples can be prepared and run on the same day. Weekly performance figures based on HPLC will therefore be available to the industry at least a day earlier than those based on GC.

(b) <u>Ease of operation</u>. There is no doubt that HPLC is a much simpler and easier technique to use than GC. Analytical skills are less demanding, with dilution and filtration the only steps necessary prior to analysis.

(c) <u>Toxicity</u>. A number of toxic chemicals are used for the GC analysis of sugars (pyridine, trifluoroacetic acid and hexamethyldisilazane). Great care must be taken, during both preparation and chromatography, to protect staff from these chemicals. When either cation or anion exchange columns are used for HPLC analysis of sugars, the reagents are relatively mild and non-toxic.

(d) <u>Cost</u>. Capital costs for either GC or HPLC are remarkably similar. HPLC running costs are generally lower than those for GC. This paper will examine this comparison in some detail.

HPLC RESEARCH AT THE SMRI

The SMRI obtained its first HPLC in 1977 and five years were spent attempting to develop a reliable method for the analysis of sugars in final molasses. Amino-bonded silica columns were initially used as they were capable of separating fructose, glucose, sucrose and the trisaccharides. Unfortunately the amino group reacted with the reducing sugars and indifferent results were obtained (6). In addition, the eluent, acetonitrile, was both expensive and toxic.

Advances in instrumentation and the introduction of the cation exchange columns in the mid-1980's rekindled interest in this procedure. Many cation exchange columns were evaluated for the analysis of sugars in a wide range of factory products. Details of

these studies were published recently (4). Satisfactory results were obtained for all products with the exception of final molasses. New sodium-based columns generally produced excellent results; howeve, overestimation of sugars occurred on older columns. HPLC running costs are based to a large extent on membrane filters and column life. The soft-gel nature of these columns limited column life and hence increased running costs. In all the above work, a refractive index (RI) detector was used to determine sugar concentrations. This detector is in principle non-specific and it is not surprising therefore that co-eluting impurities inflated sugar estimates on older cation exchange columns.

In the early 1980s, the Dionex Corporation introduced an anion exchange (AE) column for the separation of carbohydrates (1). The separation mechanism of these columns relies on the fact that carbohydrates are complex alcohols that can be ionised at high pH. The acidity of each hydroxyl group depends on neighbouring groups and different sugars have different degrees of dissociation at certain pH values. With an eluant at the appropriate pH, many sugars can be separated rapidly by high performance ion chromatography (HPIC) (2). As the ionisation of sugars is pH sensitive, these columns are notably more selective than the gel-based cation exchange columns.

In 1983 Dionex introduced an electrochemical detector to monitor sugar separations. Carbohydrates can be oxidised readily on the surface of a gold electrode. Most sugars produce a maximum current at similar applied potentials. This important property makes the pulsed amperometric detector (PAD) equally efficient at detecting reducing (fructose and glucose) and non-reducing (sucrose) sugars. A repeating sequence of three applied potentials for specific durations ensures that the surface of the gold electrode remains in its original state even after prolonged use. The PAD, besides being more sensitive, is therefore more specific than the refractive index detector (12).

Morel du Boil (7) first used the Dionex AE/PAD system at the Institute to confirm that theanderose, a trisaccharide, was present in cane products and that it was partially responsible for sucrose crystal elongation. Morel du Boil and Schäffler (8), participating in an ICUMSA collaborative study, used the same technique to measure sucrose, fructose and glucose in C-molasses samples. Thompson (11) improved the precision of the AE/PAD procedure by introducing lactose as an internal standard. This approach ensured that multiple dilutions had no serious effect on the method's precision. He also corrected for the PAD's drift by bracketing samples with calibration standards.

This paper describes the use of HPIC for the analysis of sugar in both mixed juice and final molasses. The paper compares the

technique with the official GC procedure by analysing both MJ and molasses samples over extended periods with the same deadlines and quality control parameters.

METHODS AND MATERIALS

Equipment

A Spectra-Physics SP 8880 autosampler and Spectra-Physics IsoChrom pump were coupled to a Dionex CarboPac PA1 guard and main column using sodium hydroxide (0.15M) as eluent. A Dionex pulsed amperometric detector and Hewlett-Packard 3396A integrator were used to monitor column eluants. Separations were carried out at constant temperature. An aqueous cell and a newer solvent compatible cell were used with the PAD control module. Potentials and timing intervals were those recommended by Dionex.

Mobile phase

Correct preparation of the mobile phase is critical if contamination from sodium carbonate is to be avoided. Experimental details are outlined in Appendix 1. Operating flow rate was 1 cm^3 per minute. The flow was reduced to 0,1 cm^3 per minute when the column was not in use. Column eluent was not recycled.

Sample preparation

Details for the preparation and running of samples are supplied in Appendix 2.

RESULTS AND DISCUSSION

Comparison between GC and HPIC

The South African sugar industry is in the fortunate position of having an existing, accurate method for determining sugars in factory products. This is often not the case and it is therefore difficult to assess a new or proposed procedure. HPIC was evaluated by running samples of MJ and molasses over extended periods (3 weeks), 50 samples of each stream being compared; the raw data can be found in Appendices 3 and 4. The two methods are also graphically compared in Figures 1 and 2. A statistical summary of the comparison is included in Tables 1 and 2.

The main findings of this inter-method comparison are that:

(a)   comparison for all three sugars in MJ was very good.
        Repeatabilities were virtually identical for both GC and
        HPIC for the two monosaccharides. GC precision for
        sucrose was marginally better; however, a repeatability
        of 0.15 for sucrose by HPIC is still excellent. Although
        there appears to be a marginal bias for both fructose and
        glucose in different directions, the bias was less than
        0.01 units.   Again this is insignificant.   The zero
        difference between GC and HPIC for sucrose in juice was
        most encouraging.

(b)   repeatabilities for the two chromatographic procedures
        for molasses were remarkably similar for all three
        sugars.   The precision is all the more impressive when
        one realises that these results were obtained under
        "live" conditions, with a deadline for the results
        imposed for each of the three weeks.   In addition, no
        outliers were removed during the evaluation process. Once
        again each sugar yielded a minor statistical bias between
        methods. This brcause of the extremely narrow confidence
        intervals obtained for each method and is considered to
        be of no practical significance. Greater variations have
        been found with interlab GC-comparisions (5).

Running Costs

Day-Lewis and Schaffler (4) established that GC running costs were
approximately US$ 4.00 per sample. HPLC costs can be divided into
filtration, consumables and column costs. To date, more than 4,000
injections have been applied to the same CarboPac column with no
build-up of back pressure or loss of column efficiency. This point
is extremely important as previous columns tested all tended to
deteriorate after 1000-2000 injections.   The CarboPac column
consists of rigid pellicular beads that are extremely robust, and
decontamination using 1M acetic acid followed by 1M sodium
hydroxide has been most effective in restoring column efficiency.
Running costs of HPIC have been estimated at US$ 2.00 per sample
which is significantly less than the corresponding GC costs.

The only precautions necessary are the avoidance of carbon dioxide
ingress, ambient temperature control during analysis, periodic
renewal of the injector rotor seal and the cleaning of the gold
electrode.

Analysis time

If HPIC is to become a viable alternative to GC then the total run
time should be less than that required by GC. A comparison of run

times for a typical weekly molasses run (17 samples in duplicate) is given in Table 3.

It is obvious from the table that sugar results could be available to sugar factories a day earlier if HPIC rather than GC is used. This time advantage can be especially useful at month-ends when deadlines for factory figures are critical.

CONCLUSIONS

The current HPIC system, including the Dionex anion exchange column and PAD detector has been shown to be ideally suited for the determination of sugars in mixed juice and final molasses. It compares extremely favourably with the official GC procedure with respect to precision, accuracy, running costs and run times. The SMRI intends to use HPIC instead of GC for molasses analysis for the 1992/93 season.

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Table 1. Sugars in mixed juice by GC and HPIC, a statistical comparison

Statistic	Fructose		Glucose		Sucrose	
	GC	HPIC	GC	HPIC	GC	HPIC
Mean	0.36	0.37	0.36	0.36	11.16	11.16
Min	0.22	0.22	0.18	0.19	9.76	9.68
Max	0.67	0.68	0.92	0.84	14.40	14.16
SD	0.005	0.007	0.006	0.006	0.016	0.054
r	0.014	0.019	0.018	0.018	0.041	0.149
Mean Diff	-0.01		0.01		0.00	
Intercept	0.02		0.03		0.79	
Slope	0.97		0.88		0.93	
r^2	0.96		0.97		0.90	

Table 2. Sugars in molasses by GC and HPIC, a statistical comparison

Statistic	Fructose		Glucose		Sucrose	
	GC	HPIC	GC	HPIC	GC	HPIC
Mean	6.5	6.6	4.0	3.9	31.1	31.3
Min	4.0	4.1	1.9	1.8	26.1	26.4
Max	11.1	11.0	10.0	9.7	36.5	36.3
SD	0.05	0.09	0.03	0.05	0.12	0.13
r	0.16	0.26	0.08	0.14	0.34	0.37
Mean Diff	-0.07		0.07		-0.2	
Intercept	0.18		-0.04		1.69	
Slope	0.987		0.992		0.956	
R^2	0.992		0.997		0.987	

Table 3. Comparison of GC and HPIC data for 17 molasses samples, in duplicate (time in hours)

Method	Sample Preparation	Dead Time	Chromato-graphy	Total
GC	8	16	17	41
HPIC	4	0	14	18
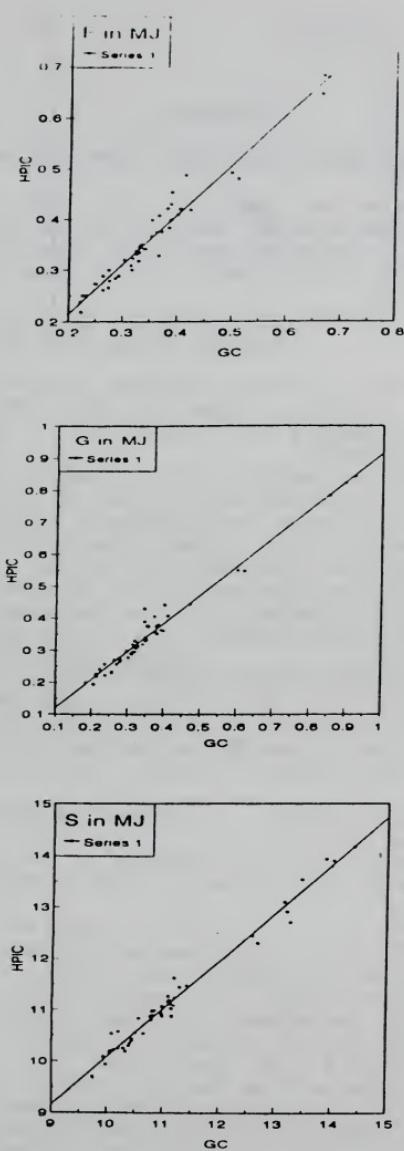
Figure 1. Comparison of sugars in mixed juice by GC and HPIC.

SPRI
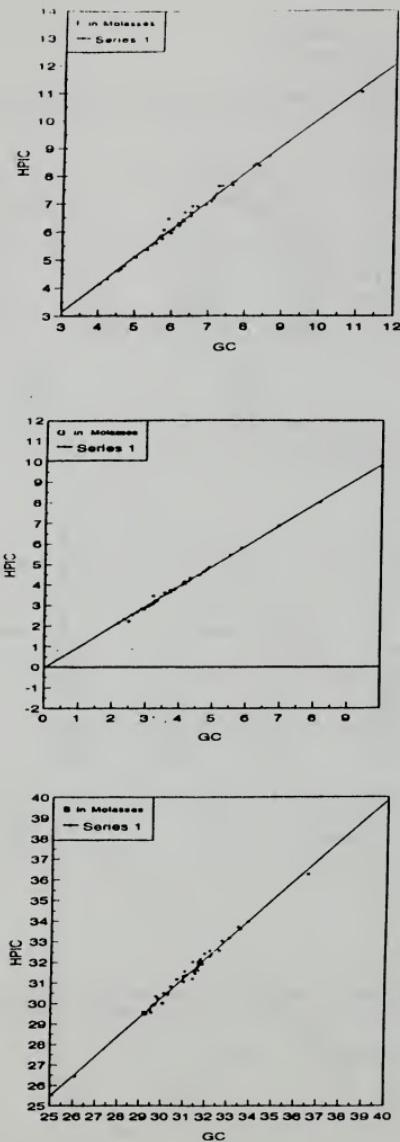
Figure 2.   Comparison of sugars in molasses by GC and HPIC.

APPENDIX 1

PREPARATION OF MOBILE PHASE FOR ANION EXCHANGE CHROMATOGRAPHY

It is essential that air (i.e. CO_2) be excluded at all times. Therefore sparging and blanketing the mobile phase with helium is necessary at **ALL** stages of eluent handling or preparation. Pyrex storage vessels seem to be superior to plastic or polythene containers.

Preparation of 50% NaOH stock solution

BDH Analar NaOH pellets (Product No 10252) and water from a Waters reverse osmosis (RO) unit are used to prepare the stock solution. Generally 300 g of 50% stock are prepared at any one time. Procedure:
(a)   Filter the RO water (0.45 μ membrane filter).
(b)   Transfer 150 ml of filtered RO water into a 250 ml Schott screw cap bottle containing a magnetic stirrer.
(c)   Stir and sparge with helium for at least 30 minutes.
(d)   Weigh 150 g Analar NaOH pellets (weigh just before use).
(e)   Add the pellets to the sparged water fairly rapidly and continue sparging and stirring until dissolved.
(f)   Allow solution to cool slightly. Remove the stirrer and sparger and close bottle.
(g)   Leave the stock to stand undisturbed for at least 5 to 7 days before use.
(h)   Do not disturb the solution at any time. Always blanket solution with helium when opened. Pipette carefully from the top clear portion. Close the bottle immediately after use.

Preparation of working eluents

It is again essential to exclude CO_2 at all times. It is important to sparge well with helium. On no account should used solvent be recycled.
(a)   Filter the RO water.
(b)   Place the required volume (e.g. 2 litres) in a Pyrex bottle fitted with a screw cap containing three small holes (i.e. for the helium sparging tube, the eluant outflow line and a vent to ensure constant blanketing without build-up of pressure). Sparge with helium for about 30 minutes.
(c)   Pipette 15.6 ml NaOH stock into 2 litres of the sparged solvent to prepare 150 mM NaOH eluant. Ensure that the solvent is sparged **continuously**.
(d)   Do not use more than about two-thirds to three-quarters of the stock sodium hydroxide. Decreasing retention times are a good indication that CO_2 contamination has occurred. It is then necessary to prepare fresh working solution and/or stock solution.

APPENDIX 2

PROCEDURES FOR PREPARING CALIBRATION STANDARDS AND MOLASSES AND MIXED JUICE SAMPLES

Lactose solution (internal standard)

Weigh 8.000 g of lactose into a 100 ml beaker. Dissolve and make to the mark in a 250 ml volumetric flask. Store the stock solution in a refrigerator. Allow the stock solution to reach room temperature before use.

Standards

Preparation of the three calibration standards is shown in the tables below.

Table 1.--Molasses Standards

	S1	S2	S3
Glucose (g)	0.020	0.060	0.100
Fructose (g)	0.030	0.070	0.110
Sucrose (g)	0.250	0.310	0.370
Lactose (ml)	5.00	5.00	5.00

Table 2.--Glucose and Fructose Solution for MJ Standards

Glucose (g)	0.25
Fructose (g)	0.25
Distilled water (g)	49.5

Table 3.--Mixed Juice Standards.

	S1	S2	S3
Glucose & Fructose Soln (g)	1.000	3.000	5.000
Sucrose (g)	0.450	0.550	0.650
Lactose (ml)	10.00	10.00	10.00

Dilute all standards to 100 ml in volumetric flasks.

Sub-divide into 10 sachets (10 ml each) and freeze.

Before each run, thaw a set of sachets (S1, S2 and S3). Dilute the standards (3 ml or 1 ml) to 100 ml for cells 1 and 2 respectively for molasses and (2 ml or 1 ml) for cells 1 and 2 respectively for mixed juice. Filter through 0.45 μ into autosampler bottles. Prepare two vials for each of the three standards, plus (x+1) bottles of S2, the prime calibration standard, where x = no. of samples.

Samples

Weigh molasses samples (1.0 g) in duplicate into 100 ml beakers. Accurately pipette 5 ml lactose solution into the beaker and then add about 50 ml of distilled water. Stir to dissolve. Transfer to a 100 ml volumetric flask and make to the mark. Dilute 3 ml or 1 ml of this solution to 100 ml for either cells 1 or 2 in a second volumetric flask. Filter through 0.45 μ into autosampler bottles.

Weigh filtered mixed juice samples (2.5 g) in duplicate into 50 ml volumetric flasks. Pipette 5 ml lactose solution into the flask and make to the mark. Dilute 2 ml or 1 ml of this solution to 100 ml for either cells 1 or 2 in a second volumetric flask. Filter as above.

Running the samples

Run the three calibration standards first to establish the system's linearity. Replicate vials from each sample are then run, sandwiched between vials of S2. Average response factors from the S2 vials bracketing the samples are used to calculate sugar concentrations for each sample. This process is continued until all the samples have been chromatographed.

APPENDIX 3

RAW DATA FOR MIXED JUICE, GC VERSUS HPIC, ALL SUGARS. A AND B ARE SAMPLE REPLICATES

MILL	FRUCTOSE GC A	B	LC A	B	GLUCOSE GC A	B	LC A	B	SUCROSE GC A	B	LC A	B
ML	0.682	0.667	0.669	0.686	0.937	0.911	0.832	0.852	9.75	9.74	9.71	9.71
PG	0.508	0.513	0.474	0.484	0.619	0.624	0.539	0.556	10.06	10.05	10.11	10.20
UF	0.363	0.370	0.330	0.327	0.393	0.403	0.366	0.357	10.39	10.40	10.29	10.32
AK	0.328	0.330	0.322	0.314	0.318	0.320	0.308	0.311	10.44	10.47	10.42	10.46
DL	0.335	0.336	0.351	0.350	0.327	0.329	0.331	0.318	11.13	11.15	11.06	11.17
MS1	0.333	0.331	0.348	0.350	0.330	0.328	0.308	0.314	11.23	11.20	11.18	11.28
MS2	0.330	0.327	0.331	0.336	0.317	0.314	0.298	0.292	10.46	10.44	10.37	10.44
ME	0.417	0.411	0.484	0.488	0.404	0.398	0.447	0.437	12.70	12.68	12.29	12.32
GD	0.274	0.275	0.305	0.296	0.284	0.281	0.267	0.271	10.66	10.66	10.49	10.59
GH1	0.264	0.263	0.294	0.283	0.265	0.264	0.272	0.267	10.30	10.30	10.29	10.23
GH2	0.328	0.332	0.332	0.339	0.313	0.317	0.316	0.321	10.57	10.59	10.81	10.86
NB	0.254	0.249	0.272	0.275	0.240	0.234	0.256	0.255	13.26	13.28	12.71	12.70
UC	0.225	0.227	0.253	0.249	0.216	0.215	0.225	0.210	13.88	13.90	13.84	14.01
IL	0.274	0.274	0.265	0.267	0.256	0.257	0.229	0.235	11.10	11.10	11.34	11.18
SZ1	0.389	0.387	0.404	0.393	0.377	0.373	0.359	0.355	10.84	10.82	10.90	10.88
SZ2	0.406	0.403	0.411	0.428	0.386	0.384	0.369	0.392	11.17	11.14	10.96	11.09
ML	0.667	0.661	0.631	0.658	0.901	0.894	0.818	0.825	9.76	9.75	9.64	9.72
PG	0.498	0.499	0.502	0.480	0.599	0.604	0.558	0.541	10.33	10.35	10.26	10.13
UF	0.355	0.349	0.364	0.369	0.381	0.377	0.367	0.383	10.87	10.85	11.05	10.93
FX2	0.400	0.400	0.407	0.411	0.412	0.410	0.403	0.412	9.94	9.94	10.08	10.11
AK	0.317	0.318	0.297	0.304	0.303	0.302	0.278	0.278	10.42	10.42	10.24	10.46
DL	0.341	0.342	0.347	0.336	0.328	0.331	0.314	0.314	11.00	10.98	10.87	10.89
MS1	0.281	0.271	0.283	0.275	0.273	0.267	0.257	0.255	10.22	10.20	10.28	10.23
MS2	0.319	0.318	0.321	0.325	0.312	0.310	0.287	0.293	10.84	10.78	10.86	10.86
ME	0.387	0.390	0.426	0.435	0.373	0.376	0.404	0.408	12.60	12.59	12.43	12.46
GD	0.315	0.315	0.308	0.310	0.316	0.309	0.290	0.286	11.00	10.99	11.06	11.07
GH1	0.292	0.294	0.291	0.286	0.278	0.279	0.265	0.262	9.98	9.99	9.95	9.93
GH2	0.356	0.351	0.401	0.397	0.349	0.345	0.392	0.389	10.98	10.99	10.93	10.89
NB	0.235	0.231	0.248	0.253	0.212	0.212	0.223	0.228	13.22	13.19	12.88	12.94
UC	0.223	0.224	0.226	0.212	0.206	0.206	0.195	0.192	14.02	14.03	13.90	13.88
IL	0.269	0.260	0.265	0.258	0.243	0.233	0.222	0.220	11.10	11.09	11.18	11.14
SZ1	0.388	0.381	0.392	0.374	0.352	0.347	0.349	0.331	11.21	11.19	11.06	11.11
SZ2	0.424	0.423	0.421	0.416	0.390	0.391	0.367	0.362	11.43	11.42	11.39	11.55
ML	0.658	0.674	0.677	0.685	0.853	0.854	0.780	0.784	10.80	10.85	10.86	10.92
PG	0.398	0.411	0.418	0.425	0.472	0.469	0.444	0.445	10.20	10.23	10.57	10.59
UF	0.325	0.351	0.351	0.333	0.327	0.356	0.319	0.318	10.50	10.53	10.52	10.59
FX2	0.385	0.385	0.385	0.382	0.381	0.381	0.350	0.354	10.97	10.97	10.94	10.96
AK	0.316	0.332	0.341	0.334	0.305	0.292	0.299	0.297	10.07	10.11	10.54	10.54
DOL	0.367	0.365	0.411	0.403	0.362	0.355	0.377	0.373	10.82	10.78	10.83	10.79
MS1	0.325	0.323	0.334	0.329	0.320	0.319	0.297	0.295	11.14	11.11	11.19	11.17
MS2	0.303	0.301	0.320	0.320	0.290	0.265	0.277	0.275	10.13	10.14	10.24	10.21
ME	0.378	0.384	0.420	0.423	0.342	0.366	0.376	0.377	13.16	13.16	13.13	13.07
GD	0.331	0.329	0.343	0.347	0.310	0.330	0.330	0.329	11.16	11.17	10.84	10.91
GH1	0.323	0.328	0.337	0.334	0.320	0.327	0.311	0.302	10.06	10.06	10.22	10.18
GH2	0.391	0.387	0.451	0.456	0.342	0.351	0.428	0.433	10.77	10.79	10.86	10.90
NB	0.249	0.248	0.277	0.271	0.221	0.225	0.240	0.239	13.47	13.46	13.49	13.57
UC	0.221	0.224	0.244	0.234	0.180	0.188	0.201	0.196	14.40	14.40	14.15	14.17
IL	0.292	0.281	0.287	0.281	0.262	0.251	0.232	0.227	11.20	11.21	11.66	11.58
SZ1	0.358	0.367	0.379	0.370	0.349	0.350	0.330	0.328	11.31	11.30	11.44	11.45
SZ2	0.370	0.371	0.382	0.369	0.352	0.352	0.337	0.329	10.82	10.82	10.99	10.97

APPENDIX 4

RAW DATA FOR MOLASSES, GC VERSUS HPIC, ALL SUGARS. A & B ARE SAMPLE REPLICATES

	FRUCTOSE				GLUCOSE				SUCROSE			
	G C		L C		G C		L C		G C		L C	
MILL	A	B	A	B	A	B	A	B	A	B	A	B
ML	9.08	9.15	9.19	9.15	8.14	8.17	8.04	8.00	31.68	31.59	31.84	31.63
PG	6.00	6.04	6.10	6.03	3.61	3.65	3.56	3.56	29.97	30.14	30.02	30.00
UF	4.76	4.67	4.73	4.83	2.62	2.61	2.53	2.58	33.25	32.82	33.05	33.28
EN	4.61	4.63	4.65	4.69	3.27	3.27	3.17	3.21	33.47	33.58	33.60	33.61
FX	6.22	6.25	6.29	6.29	4.36	4.35	4.33	4.29	30.21	30.35	30.43	30.42
AK	5.61	5.83	5.90	5.82	3.81	3.85	3.77	3.73	30.93	31.07	31.11	30.98
DL	6.49	6.52	6.64	6.71	3.74	3.70	3.71	3.77	31.48	31.52	31.32	31.69
MS	6.41	6.30	6.71	6.64	4.14	4.10	4.10	4.02	31.57	31.74	31.56	31.60
ME	7.71	7.72	7.80	7.74	4.88	4.88	4.88	4.85	31.60	31.70	32.19	31.84
GD	7.16	7.00	7.08	7.03	4.80	4.82	4.74	4.76	31.75	31.75	32.19	32.09
GH	5.72	5.84	6.06	6.06	3.48	3.44	3.40	3.42	31.70	31.70	32.01	32.11
NB	8.67	8.60	8.71	8.63	4.75	4.70	4.68	4.61	29.96	29.74	30.42	30.03
UC	7.09	7.14	7.20	7.15	2.36	2.37	2.34	2.31	29.20	29.21	29.40	29.52
IL	6.52	6.52	6.50	6.60	3.13	3.15	3.00	3.03	29.74	29.66	29.95	30.00
SZ	7.66	7.69	7.79	7.72	4.56	4.58	4.48	4.48	29.08	29.29	29.63	29.57
UK	7.15	7.14	7.16	7.19	4.12	4.07	4.01	4.03	30.95	31.00	31.32	31.29
Z	4.11	3.97	4.15	4.13	1.88	1.87	1.85	1.84	36.60	36.46	36.13	36.39
ML	8.25	8.20	8.37	8.31	6.95	6.94	6.90	6.86	32.16	32.20	32.30	32.23
PG	6.17	6.18	6.30	6.31	3.75	3.74	3.67	3.66	29.47	29.54	29.68	29.54
UF	4.55	4.54	4.61	4.60	2.51	2.51	2.05	2.40	33.96	33.80	33.94	34.01
EN	4.25	4.24	4.33	4.30	2.89	2.88	2.84	2.82	32.20	32.31	32.32	32.46
FX	6.27	6.30	6.40	6.31	4.25	4.28	4.18	4.14	30.08	30.14	30.50	30.50
AK	5.71	5.61	5.79	5.80	3.66	3.59	3.57	3.58	31.72	31.37	31.67	31.69
DL	6.03	6.05	6.27	5.95	3.34	3.36	3.30	3.16	31.30	31.52	31.35	31.00
MS	6.29	6.30	6.42	6.43	3.88	3.86	3.82	3.80	30.95	31.28	31.26	31.45
ME	7.66	7.67	7.68	7.57	4.62	4.61	4.53	4.46	32.74	32.46	32.61	32.50
GD	6.32	6.34	6.37	6.34	3.83	3.85	3.76	3.75	31.71	31.63	31.90	31.85
GH	5.73	5.75	5.74	5.75	3.24	3.24	3.09	3.12	32.19	32.14	32.68	32.39
NB	8.38	8.37	8.36	8.32	4.56	4.56	4.46	4.45	30.47	30.16	30.45	30.63
UC	6.93	6.93	6.97	6.94	2.40	2.37	2.32	2.30	29.59	29.78	29.83	30.01
IL	6.17	6.22	6.24	6.16	3.05	3.09	2.96	2.92	29.22	29.25	29.46	29.47
SZ	7.22	7.33	7.23	8.00	4.11	4.15	4.05	4.25	29.36	29.30	29.48	29.50
UK	7.13	7.13	7.10	7.23	3.86	3.89	3.78	3.86	30.65	30.74	31.11	31.21
X	11.09	11.09	11.02	10.98	9.95	9.95	9.77	9.70	26.10	26.11	26.52	26.35
ML	7.32	7.45	7.58	7.66	5.82	5.87	5.78	5.85	32.00	31.64	32.17	31.80
PG	6.27	6.31	6.38	6.33	3.82	3.85	3.81	3.72	29.60	29.50	29.51	29.61
UF	5.00	4.99	5.07	5.13	3.11	3.12	3.02	3.05	33.42	33.46	33.62	33.76
FX	6.22	6.20	6.24	6.25	4.16	4.19	4.08	4.02	30.40	30.44	30.74	30.88
AK	5.37	5.30	5.38	5.36	3.34	3.30	3.25	3.22	31.71	31.95	32.03	31.73
DL	5.59	5.54	5.58	5.58	3.22	3.21	3.08	3.06	31.93	31.95	32.38	32.41
MS	5.47	5.46	5.47	5.57	3.33	3.33	3.23	3.28	31.57	31.25	31.94	32.09
ME	7.16	7.14	7.28	7.22	4.15	4.21	4.16	4.12	32.74	32.69	33.03	33.03
GD	6.74	6.64	6.94	6.86	4.34	4.29	4.34	4.33	31.33	31.57	31.53	31.58
GH	5.03	5.03	5.11	5.08	2.75	2.77	2.67	2.69	31.83	31.70	32.23	31.88
NB	8.21	8.36	8.39	8.43	4.27	4.38	4.22	4.27	29.62	29.89	30.21	30.49
UC	7.24	7.19	7.31	7.36	2.21	2.19	2.14	2.14	29.80	29.90	30.18	30.29
IL	6.04	5.91	5.92	5.97	3.03	2.91	2.81	2.85	29.61	29.54	29.91	29.86
SZ	5.97	5.84	6.49	6.42	3.24	3.22	3.50	3.42	29.45	29.47	29.67	29.76
UK	6.49	6.62	6.89	6.91	3.52	3.60	3.63	3.63	31.00	31.08	31.33	31.80
Y	7.62	7.64	7.74	7.71	5.49	5.55	5.48	5.49	31.60	31.46	31.40	31.47

DISCUSSION

Question: My question is about the PAD detector: is it possible to use that detector also for sugars separated on a cation exchange resin?

Day-Lewis: The eluent would not be right on a cation exchange system, because a high pH background is required and this can't be used with a cation exchange column. We did try another variation: the refractive index detector after the anion exchange column. That didn't work because the high pH caustic eluent was physically destructive to the R.I. detector.

Comment from Dionex: when the eluent is not caustic, there are some additional modules that can be added post-column to raise pH and make the eluent suitable for the PAD detector, so that it can be used with other columns.

Question: Will you comment please on limits of detection and range of detection for the two techniques, GC or HPAE (IC)?

Day-Lewis: For those two techniques: with GC, you're really interested in the lower limit, because higher concentrations can be adjusted. The lower limit that we find, with a reasonable degree of accuracy, is about 50 mg/kg sucrose, because of the dilution factor in derivatization, in splitless mode, and about 500 mg/kg sucrose on sample for the split mode. With IC, about 0.5 mg/kg is the lower limit.

Question: A comment: we agree with the usefulness, precision and accuracy of IC, and we use it routinely now for analysis of invert in raw and white sugars, although we don't go much below 10 ppm.

PROPOSAL FOR THE ON-LINE UTILIZATION OF THE NIR TECHNIQUE
TO CONTROL FERMENTATIONS

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ABSTRACT

The study and the optimization of fermentation processes need an
automatic and continuous control of the fermentation parameters no
matter how these processes are carried out, whether by batch, fed-
batch, feed-back or continuous process.   At present, by using
suitable sensors on-line, we can continuously collect data concern-
ing pH, temperature, pressure, viscosity, weight, power absorption,
aeration rate, and oxygen and carbon dioxide contents in effluent
gases leaving the apparatuses.

Chemical parameters, such as substrate and nutrients contents,
metabolite formation and biomass concentration, are obtained
through laboratory measurements and fed into the computer off-line.
By following this procedure, the possibility of controlling and
optimizing fermentation is rather limited, because laboratory
analyses are relatively time consuming. Bearing in mind this fact
and the versatility of the NIR technique, we have carried out a
series of tests for the utilization of such technique for the
control of glucose fermentation in order to produce lactic acid.

Good calibration curves have been obtained for the substrate,
metabolite and biomass concentrations control. A scheme allowing
the utilization of the NIR technique for the on-line control of
these fermentations is proposed.

INTRODUCTION

In recent decades increasingly sophisticated systems of plant
automation have been developed in all sectors of industrial
production. In particular, thanks to more versatile computeriza-
tion systems, automation has recently reached such a level that
whole processes are completely operated in a centralized way. Such
centralized systems need on-line evaluations of some fundamental
process parameters and these are, in general, physical because
analyses of a chemical type can generally not be carried out on-
line.

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An improvement of automation systems could be obtained by introducing new analytical techniques which permit on-line evaluation of those chemical parameters necessary for process control. Such a problem could be solved through the utilization of the NIR technique which for several years now has been employed in the laboratory for checking the composition of a number of different products, which may be solid, liquid or doughy, and which can be applied in various industries. For some years this technique has interested the sugar industry and systems for its utilization on-line have been studied (1,2,6,9).

We consider that the NIR technique can also be usefully applied to the biotechnology area since it is increasing in importance for the utilization of renewable raw materials via biotransformation and fermentation processes.

Measurements on-line using the NIR technique have been set up for the quantitative determination of glucose in real-time, during the enzymatic hydrolysis of starch in a bioreactor (8). In the special case of fermentation processes, which can be carried out batch, fed-batch, feed-back or continuous, automatic and continuous control of all the possible fermentation parameters becomes essential.

At the moment, by using suitable sensors on-line, we can obtain in a continuous way data about pH, PO_2, temperature, pressure, viscosity, weight, power absorption, aeration rate, as well as oxygen and carbon dioxide contents of gases leaving the fermenter. Chemical parameters, such as contents of substrates and nutrients, metabolite formation and biomass concentrations are obtained by laboratory measurements and fed into the computer off-line. This fact markedly limits the possibility of controlling and optimizing fermentation processes bearing in mind the relatively long times required for laboratory analyses.

From these lines of thinking, trials were carried out in order to adopt the NIR technique to the control of a laboratory fermenter which was connected to a micro-filtration device. In particular, glucose fermentation for production of lactic acid was studied. As is well-known, such oxyacids can have a wide range of applications (4). Lactic acid can be utilized for the preparation of biodegradable polymers and for use in the pharmaceutical and biomedical fields (3,7). Without going into details about the special system which we have set up for the metabolite recovery by using an ion-exchange resin system (5), and once again applying the NIR technique, we will describe here the fermentation control.

EXPERIMENTAL

According to the scheme shown in Figure 1, a fermenter completely automated as regards control and registration of pH, temperature, oxygen concentration and weight, is fed with an artificially-prepared broth. This broth is made by using glucose as substrate plus nutrients in accordance with the list in Table 1, where the lactobacillus strain and operational conditions are also indicated. The micro-filtration system, which is operated by the fermenter computer, can, at the appropriate time, micro-filter a portion of the broth undergoing the biomass recycle. In Figure 2 the fermenter and micro-filtration plant are shown.

At the beginning of our experiments we considered the possibility of setting up NIR calibration curves for estimating concentrations of substrate (glucose), metabolite (lactic acid) and biomass in the broth. Broth samples from the fermenter were analyzed by HPLC (Aminex HPX-87H column and refractometric detector) to determine the concentrations of glucose and lactic acid. The biomass levels were determined by weighing, after filtration through a 0.45 μ Millipore filter, washing with water and drying overnight in vacuum oven at 64°C. The same sample of broth was submitted to a triple reading by NIR using a Bran+Luebbe INFRAALYZER 450 fitted with a cell for liquids (Figure 3). The parameters relating to the calibration curves are given in Table 2. It can be seen that we obtained good calibration curves even with a fairly low number of samples. The relatively high value of the standard error for the biomass calibration curve may be put down to the low reproducibility of the data coming from the manual analytical method. In Figures 4,5 and 6 the calibration curves for glucose lactic acid and biomass, respectively, are shown.

Following the favourable results obtained with these calibration curves, and having carried out a series of validation tests, we were encouraged to connect the NIR directly to the fermenter, whilst continuously passing the broth through the measurement cell by means of a peristaltic pump, according to the scheme shown in Figure 7. During each fermentation, at prearranged intervals, the readings of the three components, glucose, lactic acid and biomass, were carried out by NIR. In Figures 8 and 9 the variations of these three parameters are shown during two batch fermentations; the second fermentation was carried out under less favourable conditions than the first one.

The graph does not give the data for the first hours of fermentation, when the variations of the three parameters were negligible, because the micro-organisms were in the lag phase. Because the INFRAALYZER 450 makes readings only via manual input, it was not

possible to follow the development of the process throughout the night. Figures 8 and 9 also show some data for the above three parameters which were determined in the traditional manner in the laboratory.

The total reliability of the NIR data in comparison with the manually obtained data can be observed and with the advantage that, with the NIR technique, we can almost continuously follow the evolution of the whole process.

We have to emphasize that the particular NIR equipment used is not ideal and able to follow, under the best conditions, the whole process since, as is pointed out above, readings are made only by manual imputs. It would be necessary, according to our research programme, to use a completely automated NIR apparatus, such as the 600 D/S of Bran+Luebbe, which we have successfully employed for on-line analysis of sugar factory juices (9).

If we want not only to follow but also to operate a fermentation of a feed-back or continuous type by using the parameters given by NIR through a micro-filtration plant, we believe that it would be sufficient to interface the computer of the NIR equipment with the fermenter computer according to the scheme given in Figure 10. From the analytical data obtained by NIR, the fermenter computer would simultaneously or sequentially manage the micro-filtration modulus together with the fermenter feeding unit. Thus the operation of the whole plant would be completely automated.

CONCLUSIONS

The versatility of the NIR technique has been confirmed as also giving good results for on-line control of fermentation processes. This would permit optimization of industrial processes, thus lowering their operating costs. From the research point of view, such control would facilitate in depth studies of biotechnological transformations.

ACKNOWLEDGEMENT

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Table 1.   Fermentation conditions

Strain   _Lactobacillus casei_ DMS 2011

pH   6.4-6.6
Temperature   37°C
Anaerobic or micro-aerobic conditions
Low stirring
Glucose maximum concentration   g/100 mL   10
Yeast extract   g/100 mL   3
Magnesium sulphate   g/100 mL   0.06
Iron sulphate   g/100 mL   0.003
Magnanese sulphate   g/100 mL   0.003
Sodium acetate   g/100 mL   0.1
Potassium monophosphate   g/100 mL   0.05
Potassium biphosphate   g/100 mL   0.05
Lactic acid maxium concentration g/100 mL   8-10

Table 2.   Glucose, lactic acid and biomass: parameters of
           calibration curves

Analyte	Number of samples	Range of values	Standard error	Multliple correlation (r^2)	Number of filters
GLUCOSE	26	0-8	0.25	0.992	5
LACTIC ACID	27	0.5-9.5	0.15	0.998	3
BIOMASS	29	1-16	0.75	0.972	2
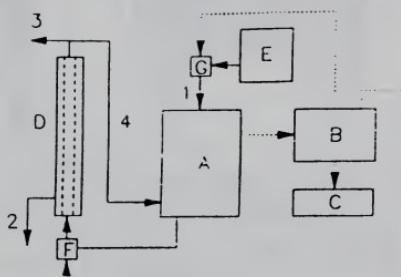
A = fermenter   G = peristaltic pump
B = computer
C = recorder   1 = feeding
D = microfiltration 2 = microfiltered broth
E = feed supply   3 = biomass blowdown
F = pump   4 = recycling

Figure 1.   Flow diagram of the fermentation cycle.
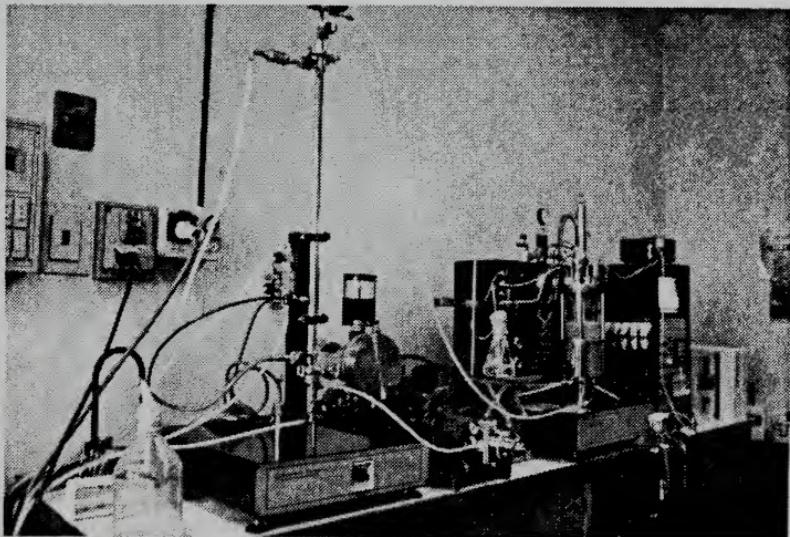
Figure 2.   The fermenter with the micro-filtration plant.

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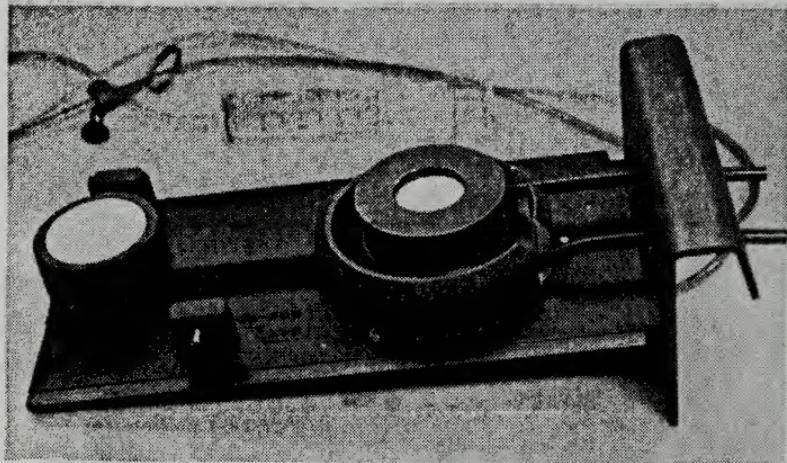
Figure 3.   The INFRAALYZER 450 with cell for liquids.
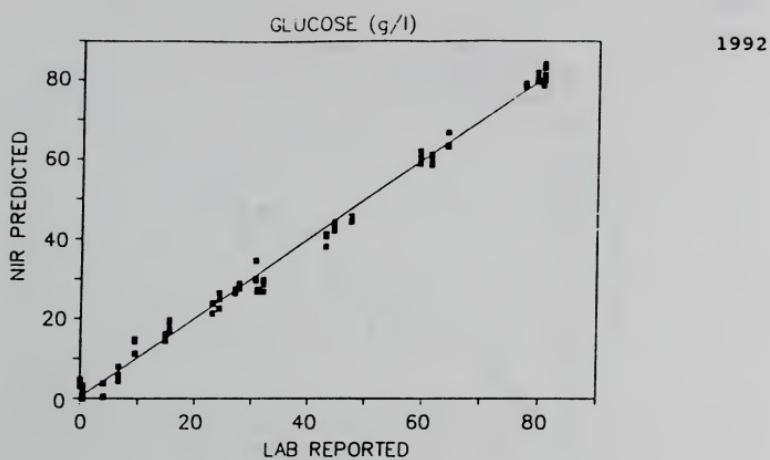
Figure 4. Glucose calibration curve.
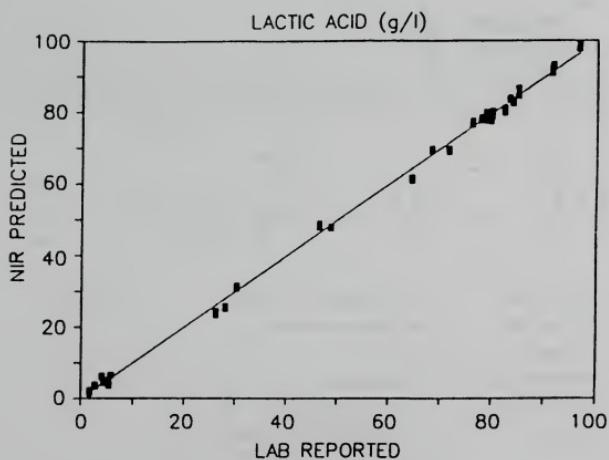
Figure 5. Lactic acid calibration curve.
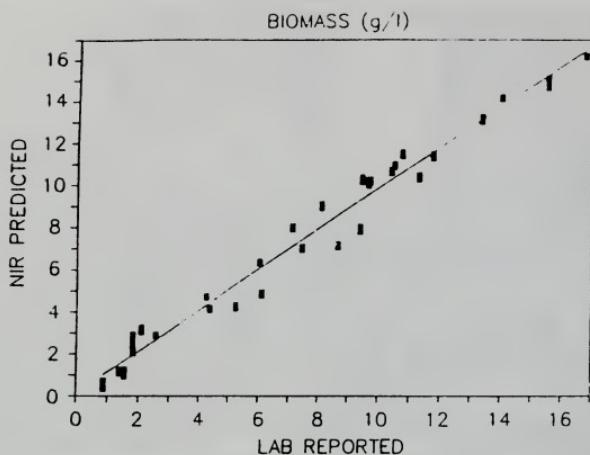
Figure 6. Biomass calibration curve.
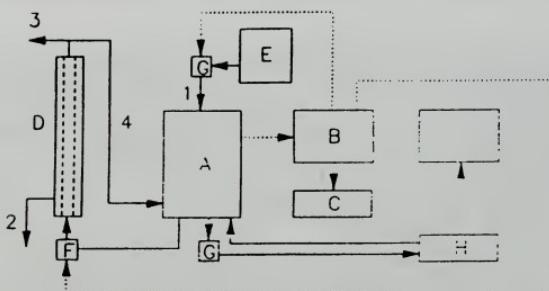
A = fermenter	G = peristaltic pump	4 = recycling
B = computer	H = measurement cell	
C = recorder	I = NIR	
D = microfiltration	1 = feeding	
E = feed supply	2 = microfiltered broth	
F = pump	3 = biomass blowdown	

Figure 7. Connection between the NIR apparatus and the fermenter.

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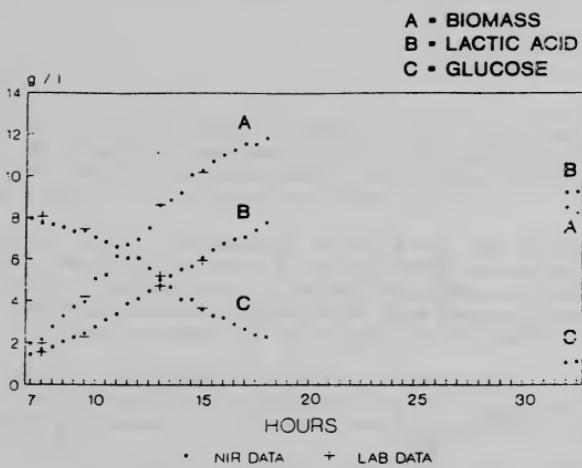
Figure 8. Changes in glucose, lactic acid and biomass concentrations during batch fermentation.
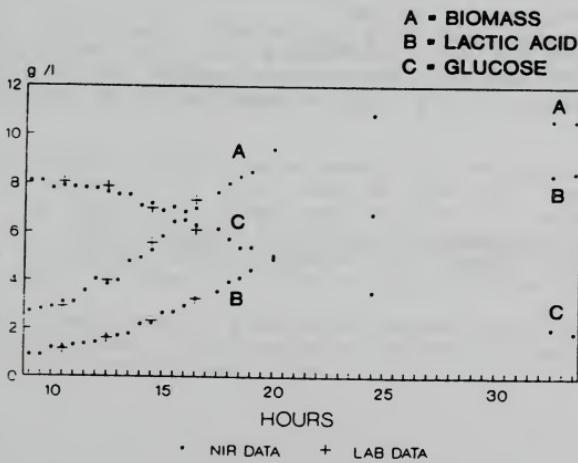
Figure 9. Changes in glucose, lactic acid and biomass concentrations during a batch fermentation carried out under less favourable conditions than in Fig. 8.
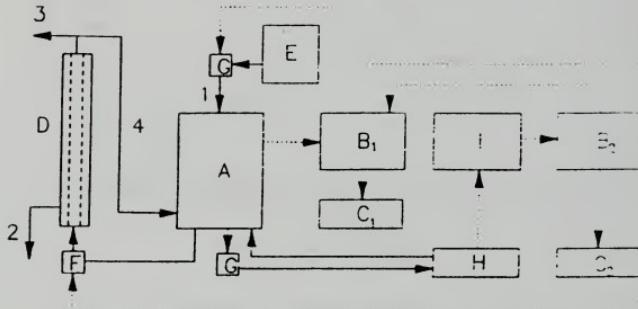
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A = fermenter   G = peristaltic pump   4 = recycling
B = computer   H = measurement cell
C = recorder   I = NIR
D = microfiltration   1 = feeding
E = feed supply   2 = microfiltered broth
F = pump   3 = biomass blowdown

Figure 10.   Interfacing of the NIR and the fermenter computers.

DISCUSSION

Question: How did you identify glucose and lactic acid in your NIR spectra? My second question is; do you calibrate on intensity only, or do you use mathematical techniques like partial least squares analysis to get your concentrations?

<u>Mantovani:</u> It is not necessary to collect the absorption spectra and identify particular wave lengths for glucose and lactic acid. The calibration curves were obtained through a calculation program based on the multiple correlation of the absorptions concerning a number of filters automatically chosen as optimum.

To answer the second question, we use intensities. The calibration curves are not shown in the paper, but of course we use these to obtain the measurement of glucose and lactic acid concentrations.

Question: The Pfizer company, in England, is using NIR spectroscopy to monitor large scale fermentations by direct reflectance spectra, through a window of the fermentor. Perhaps you might consider shooting your light source through the glass at the sample.

Question: It's very interesting here that you are measuring concentration of a solid in a solution. Can you tell us anything about the problems of measuring a suspended solid by NIR? Are there problems of concentration limit perhaps? or problems of fouling?

<u>Mantovani:</u> As far as fouling is concerned, we had no problem. As the slides showed, the best results were for lactic acid and glucose. We don't have such good results for biomass. But - nevertheless - the NIR gives an answer for many calibrations at the same time, so one can obtain a result for biomass contact, although not as accurate as those for glucose and lactic acid.

If the solid suspended in a solution is made by microorganisms as under our conditions, there are not significant problems, either of fouling or of concentration limit.

OLIGOSACCHARIDE ANALYSIS BY ION CHROMATOGRAPHY

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ABSTRACT

The oligosaccharide raffinose is a characteristic of beet sugar, as
the kestoses are of cane sugar. Systems to identify characteristic
oligosaccharides in sugar products by ion chromatography are
described.

Commercial products of honey and fruit juices are often mixed with
sugar or corn sweetener solutions. Methods to identify the
characteristic oligosaccharides of honey and fruit juices, and to
distinguish them from those of cane and beet sugar are described.

INTRODUCTION

Analysis of oligosaccharides in commercial nutritive sweeteners
(beet sugars from sugarcane and sugarbeet; starch based sweeteners)
is of importance in manufacture and utilization of sugars for the
following reasons:

1. Identification of sweetener source in product. Sugar in a
    packet, or in bulk, or sweetener in a soft drink, can be
    identified by trace oligosaccharides present.

2. Analysis of cane starch in 10-1000 mg/kg range, in cane sugar
    processing.

3. Identification of products of polysaccharide hydrolyzing
    enzymes (amylases and dextranases) in sugar products and
    process intermediates.

4. Potential identification of addition of sugars to honey and
    orange juice.

Ion chromatographic (the name is used for brevity to represent
high-performance anion exchange chromatography, HPAEC) procedures
have presented the possibility of good separation and identifica-
tion of these oligosaccharides in elution time of under 30 minutes.

*Deceased, March 1991

Tsang (11,12) has reported identification of raffinose in beet sugars,and the separation of raffinose from galactinol in beet molasses. In this paper are reported determination of raffinose, several kestoses, maltooligosaccharides in sucrose solutions, (10%-65% w/w). Application of these analyses to the above mentioned industrial problems is discussed.

EXPERIMENTAL

General - All chromatography was performed on a Dionex Model 4500i series ion chromatography system equipped with pulsed amperometric detector (PAD) and Dionex 4400 electronic integrator. Samples were introduced via a Rheodyne injector, 25 μl loop. A CarboPac PA1 column was used at ambient temperature, flow rate 1ml/min; solvent system was a combination of NaOH and NaOAc in proportion specified below for each analysis. Standard concentrations were on the order of 10 mg/l. Sample concentrations were 1-5g/l for sugars; 5% solution for soft drinks; for oligosaccharide fractions off carbon-celite columns, solids from 1 g original sample were dissolved in 200 ml water.

Solvent (mobile phase systems), are run isocratically:

1.   For raffinose only: 30 mM NaOAc and 100 mM NaOH

2.   For maltooligosaccharides: 170 mM NaOAC and 100 mM NaOH

3.   For raffinose, maltose, kestose and nystose: 40 mM NaOAc and 100 mM NaOH

4.   For glucose, fructose and sucrose only: 100 mM NaOH

Oligosaccharides were isolated from honey, orange juice and beet sugars by a modification of the carbon celite column technique (13). Beet sugars were inverted, or hydrolyzed, by heating 10 g/l, pH 4 at 98°C for 4 hrs.

Starch was hydrolyzed with Termamyl/^{RT} 120L (Novo Nordisk Inc.) and amyloglucosidase (Sigma Chemicals, from Rhizopus mold).

Cane starch was isolated from sugarcane as described by Tsang (10). Maltooligosaccharide standards were obtained from J.T. Baker (G2), Sigma Chemicals (G3) and Boehringer Mannheim (G4, G5, G6 and G7).

Enzyme digestion procedure: 2 ml of 150 mg/kg Termamyl/^{RT} solution was added to 20 ml of substrate (3.6 mg cane starch) which had been

boiled for 5 min, and cooled to 70°C. Amyloglucosidase, 20 mg, was then added, stirred 30 min, and the combined solution diluted to 1000 ml for HPAE analysis. For analysis of starch in sugars, to 10 g (white or raw) sugar, dissolved in 40 g water, heated to 100°C, 5 min, cooled to 70°C, was added 1 ml Termamyl/RT, 150 ppm solution. After 25 min, stirring, ambient temp., 10 mg amyloglucosidase and 10 ml pH 4 buffer added; stirring 20 min, ambient temp, and dilution to 1000 ml for HPAE analysis.

RESULTS AND DISCUSSION

Results for each system indicated in the introduction are listed accordingly:

Identification of sweetener source

Identification of source of sugar in packets or in bulk can be made on the basis of the presence of raffinose, at concentrations between 300 and 1200 mg/kg, as shown in the HPAE chromatogram in Figure 1. Sugars made from sugarcane do not contain raffinose at these concentrations. No cane sugar of 52 samples tested, from a variety of countries, contained observable raffinose, although traces of raffinose have been reported in sugarcane molasses (7). Identification of source is an essential factor when product quality or liability is in question. The type of nutritive sweetener in a beverage, of interest for similar reasons of product quality or liability, can be any of several: sugarcane sucrose, sugarbeet sucrose, or starch-based sweetener (dextrose, fructose (high fructose corn syrup, or other starch hydrolysis syrup) or mixtures of these. From 2.5 to 2.8 million tons (dry basis) of sweetener are used each year in beverages in the U.S. Figure 2a shows an HPAE chromatogram of a beverage sweetened with cane sugar (no oligosaccharides apparent) and Figure 2b one containing beet sugar (raffinose peak 8.35 min). Figure 3a shows a beverage sweetened with starch-based sweetener, indicated by the presence of maltose (12.5 min) and also, as reported by Tsang (12), by the raised baseline in the areas of elution of glucose, fructose and sucrose (3.9-5.5 min). The presence of maltose indicates that the starch hydrolysis product was probably high-fructose corn syrup or glucose syrup; a raised baseline in the 3.9-5.5 min area without a maltose peak indicates use of crystalline glucose or fructose.

Analysis of cane starch and products of starch and dextran hydrolases

Sugarcane contains native starch (about 30% amylose(10)) which is solubilized in processing and is present in sugarcane juice and syrup at concentrations of 100-700 ppm on solids. The increase in viscosity upon gelatinization of this starch can severely hinder process efficiency. The problem is treated by hydrolyzing starch in juice by addition of amylase (Termamyl[RT] heat stable α-amylase is the enzyme of choice), but resulting reducing sugars give false-ly high values in determination of reducing sugars produced by inversion of sucrose, and so interfere with process control.

Termamyl[RT] treatment of cane starch produces the series of malto-oligosaccharides, shown in the HPAE chromatogram in Figure 4a, with maltopentaose (G5) and maltohexaose (G6) predominating. Initial efforts to measure levels of maltooligosaccharides in cane factory process streams showed that concentrations of starch hydrolysis products were too low for detection (<10 ppm) among the sugarcane oligosaccharides (shown in Figure 4b).

However, upon addition of amyloglucosidase enzyme, maltooligosac-charides were hydrolyzed to glucose, to give sufficient increase in glucose peak on HPAE to serve as indication for amount of starch hydrolyzed. This method was found to give a relatively rapid (2 hr) analysis for starch in raw cane sugars and refined cane or beet sugars with good accuracy (correlation coefficient of 0.98 with standard method) and repeatability (CV = 5.2%) (10). Chromatograms showing glucose peaks before and after combined enzymatic hydroly-sis are shown in Fig. 5 for refined and raw cane sugars. The increase in glucose concentrations x 10% was used as concentration of starch in sugar.

Addition of sugars to fruit juices and honey

The addition of sugars to fruit juices or to honey has been a problem difficult to detect (4). Addition of cane sugar can be detected by stable isotope, ratio analysis (SIRA) but addition of beet sugars is more difficult to observe (5,6).

It has been proposed (8,9) that oligosaccharides from "beet medium invert syrup," a product claimed (8,9) to be the most frequent form of sugar adulterant, but in fact seldom made in the U.S. since the replacement of liquid sugar products by fructose corn syrups in the early 1980's, can be detected in orange juice (9) and honey (8) by HPAE procedures. The whole sample (honey, orange juice, or "beet

medium invert syrup" is subjected to HPAE analysis, using gradient system of NaOH and NaOAc, with time of 90 min. Peaks emerging between 65 and 71 minutes are claimed to be "fingerprint oligosaccharides" of beet sugars.

Figure 6a shows an HPAE chromatogram, using conditions described herein, for raffinose and kestose standards. Analysis of oligosaccharides of beet sugar, isolated on carbon-celite column as described, yields the chromatogram, shown in Figure 6b, of the oligosaccharide fraction showing raffinose to be the only oligosaccharide present. When the beet sugar sample extracted for this chromatogram is inverted, oligosaccharides, formed upon acidification by addition of fructose to sucrose, are isolated, and HPAE analysis yields the chromatograms shown in Figure 6c. Oligosaccharides were extracted from a commercial sample of liquid invert sugar, made from beet sugar; the HPAE chromatogram is shown in Figure 6d. It will be observed that the oligosaccharide profiles are qualitatively similar, differing only quantitatively.

Samples of several honeys and various forms (fresh, frozen, chilled) of orange juice were subjected to carbon-celite isolation of oligosaccharides. HPAE chromatograms showed that samples within each product group were qualitatively similar, with differences only in peak size. Typical chromatograms are shown in Figures 7a (honey) and 7b (orange juice). It will be observed that the pattern of oligosaccharides is again similar to that for beet invert syrup, or hydrolyzed beet sugar. Beet sugar added to orange juice will invert at juice pH (pH4-4.5) within 48-72 hr. Oligosaccharide profiles of inverted beet sugar are qualitatively similar to those of honey or orange juice, and therefore are not quantitative proof to show addition of beet sugar or syrup to honey or orange juice.

A recent presentation (2,3) by Concalon indicates that the concentration and temperature during inversion will determine the relative concentrations of oligosaccharides, and that these same oligosaccharides are further formed in orange juice when it is heated. Concalon points out that the presence of oligosaccharides in fruit juices is not necessarily an indication of the addition of beet sugar. Our observations verify Concalon's conclusions.

Identification of the remaining oligosaccharide peaks continues.

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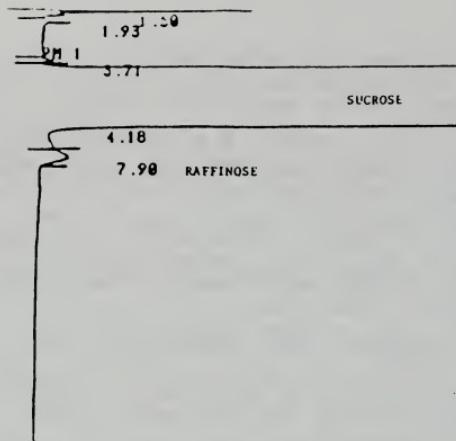
Figure 1. HPAE of white sugar containing raffinose (a beet sugar).
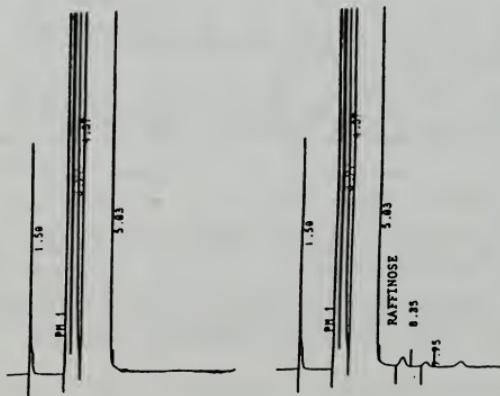
Figure 2. HPAE of beverage sweetened with (a) cane sugar (b) beet sugar, showing raffinose.

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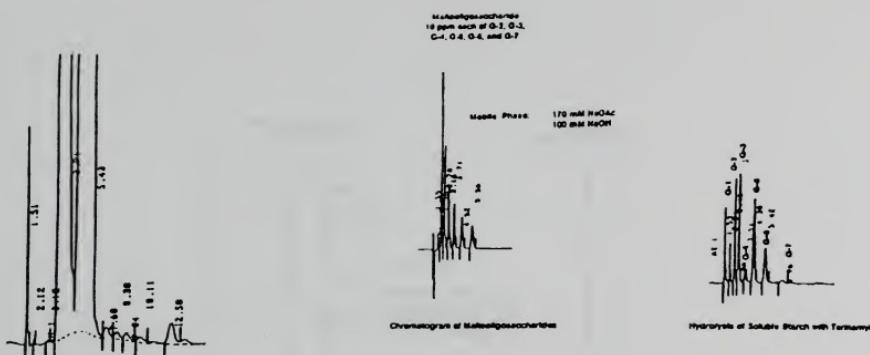
Figure 3. HPAE chromatogram of sugars in a lime-lemon drink (5%). Peaks: Invert (glucose + fructose) (3.91), sucrose (5.43), maltose (12.50).

Figure 4a. Maltooligosaccharides
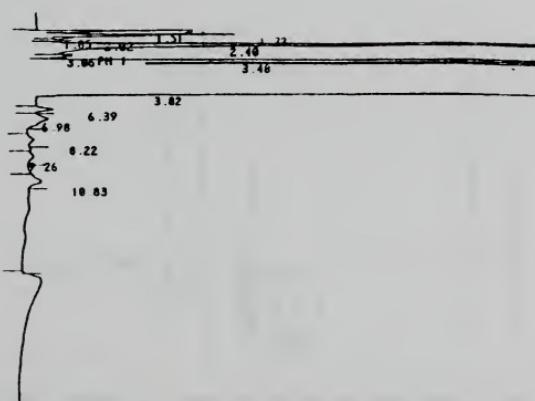
Figure 4b. Chromatogram of whole sugarcane juice.

227
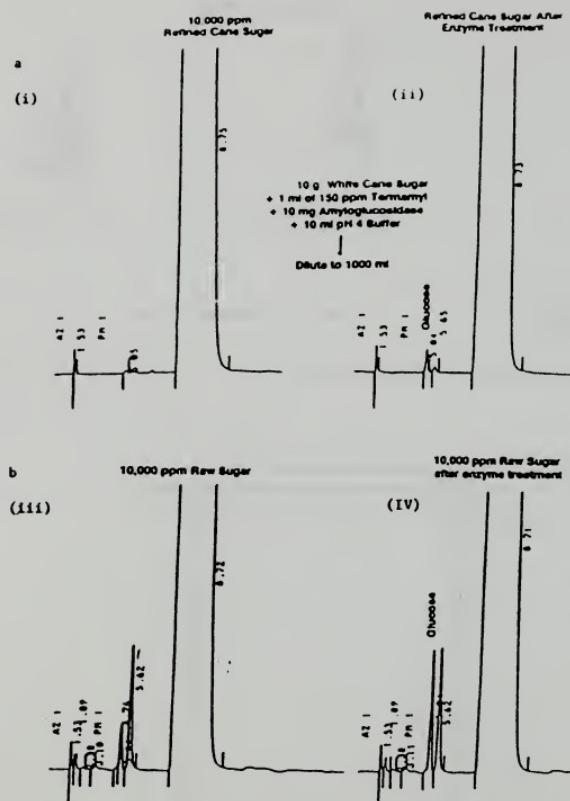
Figure 5. a. HPAE of white sugar before (i) and after (ii) enzyme treatment

Figure 5. b. HPAE of raw sugar before (iii) and after (IV) enzyme treatment
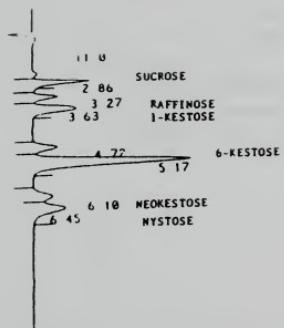
Figure 6a. Authentic samples of sucrose, raffinose,
kestoses and nystose.
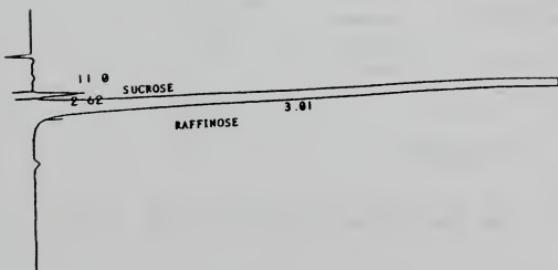
Figure 6b. Oligosaccharides from beet sugar.

SPRI
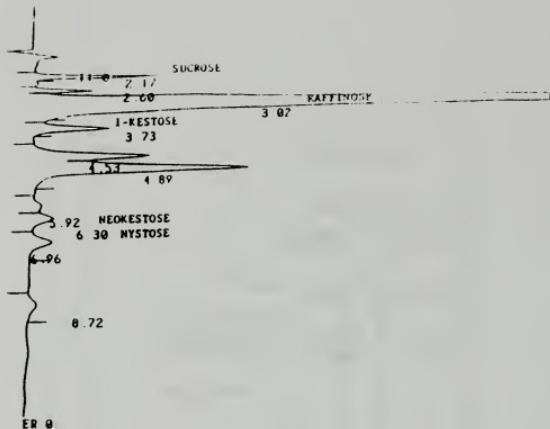
Figure 6c. Oligosaccharides from beet sugar in (b), inverted.
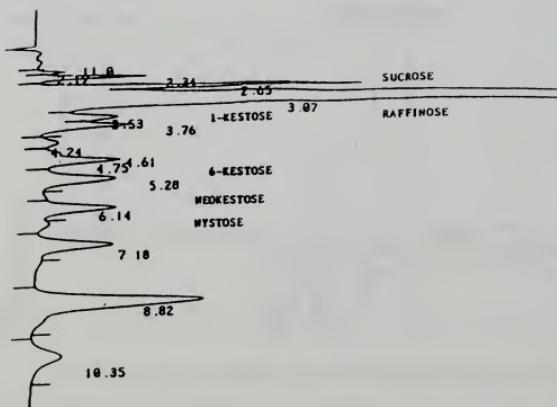
Figure 6d. Oligosaccharides from medium invert syrup (beet).

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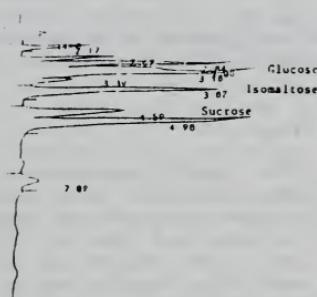
Figure 7a. HPAE of oligosaccharides of a typical honey.
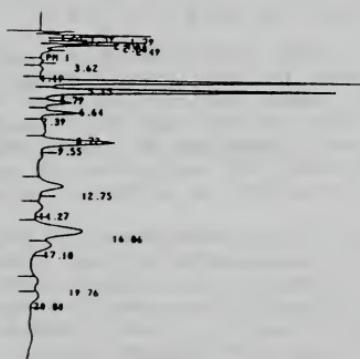
Figure 7b. HPAE of oligosaccharides of cold-pack orange juice
(Mobile phase: 30 mM NaOAc and 100 mM NaOH).

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DIETHYLAMINOETHYL BAGASSE AS A DECOLORIZING AGENT IN SUGAR
PROCESSING

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ABSTRACT

It has been found that diethylaminoethyl (DEAE) bagasse is an
excellent agent for the removal of color and turbidity from sugar
solutions.   In this paper we give a brief description of the
preparation of DEAE bagasse and its ability to remove color from
various sugar solutions.   The extent of color removal from sugar
syrups and liquors, operating conditions of the column, regenera-
tion of the DEAE bagasse for reuse,and durability of the material
with continued use will be discussed.

INTRODUCTION

Diethylaminoethyl (DEAE) chloride reacts with polysaccharides in
the presence of base to form stable compounds through an ether
linkage.   Some of these derivatives have been found useful in
certain areas.   DEAE starch has been used to improve pigment
retention in the wet formation of papers (5).   Bobleter (1) and
Petrova (2) found uses for cellulose or sawdust, respectively,
reacted with DEAE chloride, as a chromatographic support.

Several years ago, while attempting to purify a polysaccharide
isolated from cane molasses,the authors found that DEAE cellulose
was effective in removing the color from a solution of the
polysaccharide.   DEAE cellulose was then tried as a decolorizing
agent for raw sugar solution.   it was found to remove 85% to 95% of
the color and turbidity from the raw sugar.

Since sugarcane bagasse, a fibrous byproduct of cane sugar produc-
tion, is 40% to 60% cellulose (dry basis) it was of interest to
prepare a complex of DEAE with bagasse, and to study its effec-
tiveness at removing color and turbidity from sugar solutions,
syrups, and juices.

This paper describes the preparation of DEAE bagasse and its
performance in removing color and turbidity from sugar solutions.

EXPERIMENTAL

Preparation of DEAE bagasse

Whole bagasse, 30g, ground to pass a 20 mesh screen was suspended in 500 ml of water. Sodium hydroxide 10g was added. The mixture was stirred and when the sodium hydroxide was dissolved 10g of DEAE chloride hydrochloride were added and the mixture was heated to 90% for 1 hr. It was then allowed to cool and 10g of sodium hydroxide and 10g of DEAE chloride hydrochloride was added and the mixture was heated to 90°C for one hour. It was again allowed to cool and 10g of DEAE chloride hydrochloride was added and the mixture was again heated to 90°for one half hour. The mixture was then filtered with suction and the insoluble material was washed with water until no more color was removed. The DEAE bagasse was air dried. Yield 22g.

Regeneration of DEAE bagasse

After several (up to 20) decolorization treatments, DEAE bagasse was regenerated with 5% sodium chloride solution, approximately 100ml to 10g bagasse, in a single pass followed by water washing (100 ml).

TRIALS AND RESULTS

Batchwise decolorization with DEAE bagasse

Experiment I:

Five 100g batches of 25 Brix solutions of a single raw sugar were prepared. DEAE bagasse, 2g, ground to pass a 20 mesh screen and containing 1.23% nitrogen (by elemental analysis) was added to one solution and stirred for 5 minutes. The DEAE bagasse was filtered off and was regenerated by washing with 5% NaCl solution and reused. The same 2g of DEAE bagasse was used to decolorize all five samples. Colors of the treated solutions and color of the original were measured at pH 7. The results are shown in Table 1.

Column decolorization with DEAE bagasse

Experiment II: Column decolorization, single pass

A column, 48mm i.d., 60mm b.d. depth) was prepared containing 10g of DEAE bagasse which had been ground to pass a 20 mesh screen and contained 1.17% nitrogen. Several 100g samples of 40 brix solutions of different raw sugars were passed through the column.

The column was regenerated with 100ml of 5% sodium chloride after the passage of each sugar solution. The color in the effluents along with that in the original solutions were measured. It was found that the DEAE bagasse removed 85% to 90% of the color and the solutions contained no visible turbidity. The DEAE bagasse was therefore shown to have removed most of the color from raw sugar solutions; its durability under repeated use was then tested. Twenty (100g, 40 Bx) solutions of a raw sugar were passed through the column as described above followed by 200 ml of water. The column was regenerated after the passage of each solution. The color in the effluents, 200ml from each sample, along with the original solution were measured at pH 7.0. None of the effluents contained any visible turbidity. The results are shown in Table 2.

Experiment III   Turbidity removed from raw sugars

Ten 100g batches of 40 Brix solutions of different raw sugars were passed through the column of experiment II. Color and turbidity were determined in the original solutions and the effluents. The results are shown in Table 3.

Five 100g batches of 40 Brix solutions of the same sugar were passed through the column in experiment II in succession, without regeneration. The color and turbidity in the original solution and each 200 ml effluent were determined. The results are shown in Table 4.

Experiment IV: Column decolorization, multiple pass.

Five 100g batches of 40 Brix raw sugar solution were passed through the column in experiment II in succession without regeneration to determine the capacity of the DEAE bagasse for removing color. The color of the turbidity free effluents were measured along with that of the original solution. The results are shown in Table 5.

Experiment V: Column decolorization, single pass, color component, ash and polysaccharide removal.

One hundred (100g) grams of 40 Brix solutions of five raw sugars were passed through the DEAE bagasse column described in experiment II. The column was operated under gravity flow at the rate of 20 ml per minute. The effluents from the column along with the original solutions were analyzed for conductivity, ash, phenols, dextran and total polysaccharides. The results are summarized in Table 6.

Experiment VI: Column decolorization; large size, heat-jacketed column.

A jacketed column containing 20g of DEAE bagasse, ground to pass a 20 mesh screen and containing 1.11% nitrogen was prepared. The DEAE bagasse bed was 60mm in diameter and 90mm deep. A sample (500g, 40 Bx) of raw sugar solution was heated to 80°C and passed through the column under gravity flow while maintaining temperature at 80°C. The flow rate was 20ml per minute. The effluent was collected in 100ml fractions and the color in each fraction along with that in the original sample was determined. The results are shown in the Table 7.

Experiment VII: Column decolorization under pressure, refinery liquors

A column was prepared containing 50g of DEAE bagasse which was ground to pass a 20 mesh screen and contained 1.3% nitrogen. The bed of DEAE bagasse was 3 inches in diameter and 3-1/2 inches deep. A 1000g (80 ml) quantity of 60 Brix solutions each of raw sugar,melted washed raw sugar, clarified liquor, remelt liquor, and clarified remelt liquor were each heated to 80°C and was passed through the column maintained at 80°C. The column was regenerated after the passage of each solution. The flow rate was 20 ml per minute under 2 pounds of pressure. The color in each effluent (1600ml) along with that in each original solution was determined. The results are shown in Table 8.

DISCUSSION OF RESULTS

Synthesis of DEAE bagasse

Diethylaminoethyl chloride is supplied as the hydrochloric acid salt or hydrochloride. The hydrochloride is a crystalline solid which is water soluble. The reactions which occur in the preparation of DEAE bagasse are illustrated in Figure I.

In equation 1 the sodium hydroxide neutralizes the hydrochloric acid forming the DEAE chloride free base. This is a liquid and is insoluble in water but when stirred in water it rearranges to water soluble diethylaziridinium chloride (4) as shown in equation 2. This form of the reagent is ionic and is highly reactive to hydroxyl groups in the presence of base.   The reaction occurs principally at the 6-0-hydroxyl group of the glucose units (3). Sugarcane bagasse is 40% to 60% cellulose, dry basis.   The remainder is principally xylan and the lower molecular weight fraction is dissolved by the sodium hydroxide during the reaction. This accounts for the yield of 60% to 70% in the preparation of

DEAE bagasse. The DEAE-ether linkages are very stable and can only be removed under extreme conditions.

DEAE bagasse is an anion exchanger and swells when placed in water. For this reason it should be stirred in water for 20 to 30 minutes before pouring into a column. In order for it to be effective in removing color and turbidity it should contain a minimum of 1% nitrogen.

The color removed from the sugar solutions reported ranged from 72% to 95%. None of the effluents contained any visible turbidity, as indicated in Table 4.

The small amount of color not removed on DEAE bagasse was analyzed by gel permeation chromatography and shown to be low molecular weight, approximately 30,000 daltons. Colorant of this lower molecular weight range is loss likely to be occluded in the crystal. All of the very high molecular weight color (2×10^6) daltons) and 90%-95% of the major colorant at least 50,000 daltons, are removed from melt liquor by DEAE bagasse. The very high molecular fraction is difficult to remove by other adsorbents. The color adsorbed on the DEAE bagasse cannot be washed off with water, but a 5% solution of chloride, as sodium chloride, displaces the color and subsequent washing with water prepares the column for reuse. Repeated use of the DEAE bagasse does not affect its ability to remove color. The mode of action of this material in removing sugar colorants is apparently not a simple ion exchange reaction, but possibly a combination of ion exchange and gel permeation. The removal of turbidity is apparently accomplished by physical adsorption. Many of the suspended particles are charged, and so able to be adsorbed on the negatively charged, or negatively polarized, DEAE sites. No significant ash removal has been observed indicating that removal of turbidity is by adsorption and not by ion exchange.

SUMMARY AND PLANS

A complex of diethylaminoethyl anion with sugarcane bagasse (reacted with the cellulose therein) has been shown to remove colorant, polysaccharides, and turbidity from raw sugars and refinery liquors over a range of temperature and concentrations.

Plans include on-site factory trials for continuous decolorization of syrups and juices.

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Table 1. Color removed from raw sugar by single batch treatment

Sample	ICU	% color removed
Original solution	6419	-
Treated solutions		
1	979	85
2	958	85
3	964	85
4	915	86
5	878	86
Average color removal		85.6%

Table 2. Decolorization of repeat (20) batches on DEAE-bagasse column

Sample No.	ICU	% color removed
Original	2478	-
1.	492	80
2.	324	87
3.	304	88
4.	306	88
5.	287	89
6.	338	86
7.	307	87
8.	295	88
9.	337	86
10.	322	87
11.	300	85
12.	369	85
13.	356	85
14.	350	86
15.	367	85
16.	338	86
17.	307	87
18.	337	86
19.	356	86
20.	362	85

Table 3.   Turbidity and color removal.

Sample No.	Color ICU	% Color removed	Turbidity ICU	% Turbidity removed
1. Original	5384		2073	
Column effluent	368	93	85	95
2. Original	3612		1604	
Column effluent	462	88	32	98
3. Original	5595		1514	
Column effluent	557	90	97	93
4. Original	3422		1509	
Column effluent	576	83	122	92
5. Original	5339		1488	
Column effluent	586	89	55	96
6. Original	8491		9683	
Column effluent	1560	81	213	97
7. Original	3359		590	
Column effluent	470	86	62	89
8. Original	4112		1092	
Column effluent	445	89	101	91
9. Original	7949		1130	
Column effluent	502	90	73	94
10. Original	5230		1588	
Column effluent	579	88	60	95

Table 4.   Turbidity and color removal, multiple pass.

Run No.	Color ICU	% color removed	Turbidity ICU	% Turbidity removed
Original	6827		1488	
1	586	92	55	96
2	1302	80	119	92
3	1328	80	105	92
4	1847	73	107	92
5	2277	67	214	85

Table 5. Column decolorization, by DEAE bagasse, multiple pass.

Run No.	ICU	% color removed
Original solution	2478	-
1	262	89
2	572	76
3	944	62
4	1106	55
5	1204	50

Table 6. Removal of non-sugars by DEAE bagasse columns.

Sample	Cond. Ash %	Phenolic PPM	Dextran PPM	Total P'sacc. PPM
1. Raw	0.51	896	339	1340
Column effluent	0.55	352 (60)*	161 (53)*	564 (58)*
2. Raw	0.22	447	430	1805
Column effluent	0.15	175 (61)*	291 (32)*	751 (58)*
3. Raw	0.14	988	520	2599
Column effluent	0.47	352 (64)*	145 (72)*	1116 (57)*
4. Raw	0.24	641	368	1118
Column effluent	0.36	227 (64)*	109 (70)*	497 (58)*
5. Raw	0.39	696	224	1137
Column effluent	0.32	232 (66)*	-	622 (46)*

*Indicates percent removed by DEAE bagasse

Table 7. Decolorization on jacketed pressurized column at 80°C

Fraction No.	ICU	% color removed
Original solution	2826	-
3	181	93
4	194	93
5	311	89
6	420	85
7	569	80
8	736	74
9	932	67
10	1011	64

Table 8. Column decolorization, refinery liquors.

Sample	ICU	% color removed
Raw sugar	4798	-
Column effluent	540	85
Melted washed raw sugar	1197	-
Column effluent	90	92
Clarified liquor	1155	-
Column effluent	278	72
Remelt liquor	5302	-
Column effluent	1206	77
Clarified remelt liquor	3904	-
Column effluent	1075	72

Figure 1.

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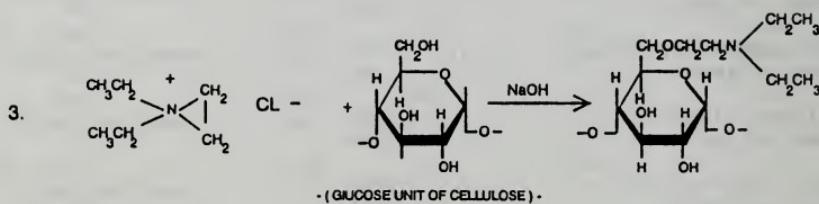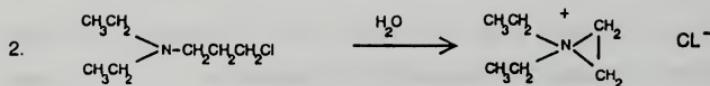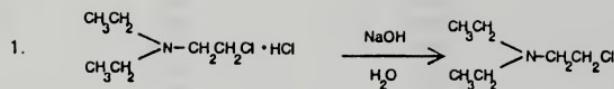
DISCUSSION

Question: Two questions: do you have the molecular weight of the dextran that was removed - was it a commercial dextran?

Roberts: No, that was normal cane dextran, that is usually found in sugar, of high molecular weight, about 1-2 million daltons.

Questions: The second question: to you think it's possible that, in the preparation of DEAE-bagasse, you are getting reaction at some of the lignin phenolic sites as well as at the cellulose sites?

Roberts: I doubt that any lignin was left after all the treatments, but it's a possibility.

Question: Is there a pH change across the column, between ingoing and outgoing liquids?

Roberts: The pH of the effluent is between 7 and 7.5.

2 ₵5

NEAR INFRARED (NIR) ANALYSIS IN SUGAR FACTORIES

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ABSTRACT

Near infrared (NIR) analysis methods have been developed for
sugarcane juice, bagasse, and cane fiber. Components analyzed in
cane juice include pol, Brix, purity, sucrose, invert, glucose,
fructose, dextran and total polysaccharides. Components analyzed
in bagasse and whole sugarcane fiber include sugar (pol), moisture
(water) and fiber (direct analysis). Results are compared to those
from standard methods. Initial work on sugarbeet juice, molasses
and brei is outlined.

An NIRSystems Model 6500 scanning instrument, with transporter
mechanism and capability for both transmission and reflectance
modes, was used for all measurements.

Personnel requirements, cost and simplicity of operation are
discussed. The status of universal calibration curves is consid-
ered.

INTRODUCTION

Near infrared (NIR) analysis is used for quality control and
product evaluation in the grain, cereal, forage and fiber indus-
tries. It is finding increasing application in quality control of
chemicals, pharmaceuticals and food products, including sugar
containing foods and dry mixtures (14,21,22). The possibilities of
near infrared analysis have been studied in several areas of the
sugar industry; there are current applications in several areas in
both the cane and beet industries. Early studies were made on
whole sugarcane, shredded, by Sverzut (17), who found satisfactory
correlation of NIR analyses with standard laboratory analyses for
pol, fiber, sugar and moisture. Wood and coworkers in South Africa
found NIR analysis for nitrogen in sugarcane leaves to be rapid and
accurate (17). In studies on analysis of Brix, pol and sucrose in
sugarcane juice, however, they concluded that, although NIR was
rapid and required no sample clarification, it was not accurate
enough for payment purposes (12). Samples were filtered but not

otherwise treated. The same group tried direct NIR analysis of shredded cane for sucrose, dry matter and fibre, but found results not as satisfactory as those for juice analysis. All samples were run in reflection mode, using multiple filter instruments.

A study at the Australian Bureau of Sugar Experiment Stations used a 19-filter instrument, in reflectance mode, on sugarcane juice and whole fibrated cane (3). The purpose was to develop rapid analyses for Brix, pol, purity, recoverable sugar fibre, moisture and conductivity in cane breeding selection studies. Problems with sample homogeneity in fibrated cane, and temperature of flow-through cell for juice, were encountered, but data were sufficiently promising to expand the study and acquire scanning NIR equipment.

Another Australian study (1) on raw sugar used a 20-filter instrument to measure pol, moisture, ash, reducing sugars in raw sugars. Calibrations were developed in an analytical laboratory and used on-site at a factory. Results were satisfactory for process control, but the unresolved question of bias led the authors to conclude that the analyses could not yet be used for payment. Similar conclusion were drawn in a subsequent paper from the same group (13) on further raw sugar studies using a slow scanning instrument or a filter instrument. Studies on molasses and fermentation broths for sugar showed promise. The authors pointed out that error in calibration of raw sugars from a single mill was less than error in an overall laboratory calibration.

A successful application of the NIR technique to analyses for moisture, starch and invert sugar in powdered and agglomerated sugars was reported by Stevens (16). The slight increase in error was more than compensated by the time and labour savings over the tedious traditional methods.

It is interesting to observe that in whole shredded sugarcane studies, only the work of Sverzut (17) found satisfactory data. Sverzut's study was performed at the Southern Regional Research Center (site of the senior author's laboratory) using a scanning instrument that is a forerunner of the scanning instrument used in the study reported herein. Standard laboratory tests on samples in the Sverzut study were performed in the senior author's laboratory.

Studies on application of near infra-red to sugarbeet juice and beet sugar products have, in general, yielded more positive conclusions. Vaccari and Mantovani and co-workers have, in work related in a series of papers (18,19,20) established NIR analysis for on-line process control on raw, thin and thick juices in the beet factory. Vaccari et al. found, encouragingly, that calibrations set up for one factory could be used for others. A filter instrument (20 filters) in reflectance mode was used. Although Vaccari et al used a smaller number of samples than other workers

to set up calibration curves, the standard deviations were comparable to those of laboratory measurements.

In France, Burzawa et al, using a 9-filter near infra-red instrument in reflectance mode, have developed a satisfactory on-line control for Brix, pol and priority in beet factories (4,5).

In the U.K., British Sugar plc have installed an on-line NIR measurement, using an NIRSystems 6500, with fiber optics probe, to determine % solids and purity on thick juice and on high green syrup (11). Results are reported to compare favorably with laboratory analyses, at a greatly increased frequency of analysis, and with no increase in labor. The instrument has proved robust in the hostile environment of the factory.

Initial work on sugarcane juice, for pol, Brix and purity analysis on samples from two geographical regions (Louisiana and Florida) over a two year period, indicated that a single calibration curve could be constructed, with data from both regions and years, that could be used for analyses of samples from either region or time, i.e. a universal calibration curve (7).

Additional sugarcane factory work on bagasse analysis for pol and moisture was reported subsequently (8). Raw sugar analyses, in two forms, dilute solution (using transmittance) and solid crystalline (using reflectance), for color and the time consuming analyses for total polysaccharides, dextran and starch, were also reported (8). Initial results indicated that the solid crystalline sugars gave better reproducibility and accuracy than the dilute solution form. Recent work in this area is reported by Edye et al (9).

The extensive potential (and actual, in the case of British Sugar (11)) uses for process control in sugar factories and refineries were presented at a Symposium on Process Sensors and Controls at the 1992 AOAC meeting (6).

CONDITIONS FOR CURRENT STUDY

The benefits of using NIR analysis include:

1. Speed: The entire NIR analysis of a liquid or solid sample can be done in under two minutes.

2. No sample preparation is required for cane or beet juice or syrups, bagasse, cane fiber, beet or cane molasses or beet brei. No chemicals, filtration or extraction are used.

3. A sample can be analyzed for any component for which a calibration curve has been run on the NIR instrument. For example, an NIR analysis on cane juice can yield results for:

Brix, pol, purity, invert, sucrose (HPLC), ash, color, polysaccharides, dextran and starch, if a calibration curve for each component has been prepared.

The juice analyses on NIR takes less than 2 minutes ad indicated above, and a further few minutes are required to call up the desired calibration curves on the computer and read out the component values for a series of samples..

It is important to emphasize the differences in conditions for the current project compared with earlier work. These conditions, the authors believe, have enabled current results in cane factories to show great improvement over earlier work.

1. Use of the scanning spectrophotometer that analyses continuously, at each 2 nm, over the wavelength range used, giving some 700 individual readings in a continuous spectrum for the 1100-2500 range, used for most analyses. Spectra for color analyses are obtained over the complete 400-2500 nm range. The choice of wavelengths at which there is an NIR absorption band is therefore much greater than in an instrument with only a few wavelengths. Greater accuracy of measurement, and an extended number of components that can be measured, are the results.

2. The continuous spectrum can be normalized (converted to a second derivative spectrum); individual wavelengths cannot be. Normalization reduces baseline scattering caused by suspended solid particles in the liquid sample. The derivative spectrum shows sharper changes in absorption (and therefore in component concentration) than does the spectrum itself. Accuracy of measurement can be increased by an order of magnitude. Most earlier work employs the direct spectrum; the second derivative permits enhancement of weak absorptions, and greater selectivity.

3. Sample presentation in the NIRSystems 6500 model used in this study moves the solid sample (liquid cells are static) across the detecting beam in a Sample Transport Module, averaging out errors caused by suspended solids in liquid samples, or different particle or fiber sizes in solid samples. This presentation makes feasible the analysis of unfiltered cane or beet juice (even diffusion juice) and syrups, and of brei, bagasse, cane fiber, solid crystalline sugar and molasses.

4. The computer averaging software package, NSAS, which derives and employs a normalized curve of the spectrum, uses recent developments in chemometrics. These further reduce variation in sample presentation, and make feasible the choice of multiple wavelengths for correlation. The software includes possibilities for partial least squares, linear least squares

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and principal component analyses, in addition to the standard regression that proved adequate for the studies reported here.

METHODS AND MATERIALS

NIR Analyses

NIRSystems Model 6500 scanning NIR spectrophotometer (NIRSystems, Inc.) was used, with Sample Transport Module. A 1 mm quartz cuvette cell was used for juice samples, and a large cell for coarse samples, with about 60 cm^2 surface area of sample, for bagasse and cane fiber samples. The instrument with the bagasse fiber sample cell is shown in Figure 1. Molasses and brei were measured in 20 mm quartz cuvette, in reflectance mode.

A table top or notebook computer was used to store and handle data. Several different models were used at the various locations. The computer must be at least a 386SX model with math co-processor chip.

Samples were scanned for 1100-2500 nm, using the lead sulfide detector, at an average of 32 scans per minute for cane juice, 50 scans per minute for beet factory samples. NIRSystems "NSAS" software package was used for all calibrations and predictions (analyses). Calibrations were developed using "conventional" NIR technology procedures, including selection of optimum wavelengths for log 1/R, or log 1/T and first and second derivatives thereof by forward stepwise regression.

Laboratory analyses

Samples of cane juice were analyzed at Sugar Cane Growers Cooperative of Florida, in Belle Glade, Florida, in January and March of 1991 and January, 1992, and at Okeelanta Sugar Co., South Bay, Florida in February 1992. Grower's samples were used in the above factories,and at M.A. Patout and Son, Jeanerette, Louisiana in November, 1991. Juice samples from variety developmental programs were analyzed at the USDA Agricultural Research Service's Sugarcane Laboratory, Ardoyne Farms, Louisiana, in October-December 1990, and November-December 1991.

Samples of beet diffusion and thin juice, brei and molasses were analyzed at Holly Sugar Corp. (Division of Imperial Holly Corporation) factories at Hamilton City, CA and Tracy, CA in September, 1992.

Juice samples were not filtered or clarified with chemicals or pretreated in any way, but simply pipetted (micropipet or eye

dropper with rubber bulb) into a 1 mm quartz cuvette cell and inserted into the NIR sample holder. All standard laboratory analyses (for pol, Brix, purity, nitrate and for sucrose and moisture on bagasse) were run by factory or USDA personnel in their normal manner. Results from these standard tests were used to generate the calibration curves. In standard procedures, pols were run at the Florida factories using Membrex filtration and no chemical treatment on juice; pols at the Louisiana locations were run using an aluminum hydroxide chemical clarification. Standard bagasse oven drying for moisture,and Blendor extraction for pol, procedures were used at the Florida factories. Beet samples were analyzed by standard procedures of the California Beet Growers' Association, using aluminum sulfate clarification for juice clarification.

Fiber analyses (in whole sugarcane, from crusher mill) were run on true duplicate samples on NIR, in the large sample cell for solids. They were analyzed for fiber according to the laboratory method used at the USDA-ARS Sugarcane Field Station, Houma, Louisiana. In this method, duplicate samples are weighed into tared linen bags, washed through 2 cycles on a standard household washing machine, and dried to constant weight.

HPLC analyses for sucrose, glucose and fructose in cane juice were run on samples from the USDA Sugarcane Laboratory, Ardoyne Farms, Louisiana, using a Sugar Analyzer (Waters Associates, Div. of Millipore Corp.) liquid chromatograph, HPX87-C cation exchange column (BioRad Corp.) in calcium form at 85°C, with 40 mM calcium acetate as mobile phase. Replicates from maturity tests and cold tolerance tests were used. Dextran laboratory analyses were run by the Roberts method (AOAC method for total dextran in raw sugar (2)), modified for cane juice by using 10 ml of cane juice as sample, and adjusting the calculation accordingly, employing the weight of solids per volume of juice.

Total polysaccharides were analyzed using the SPRI method (15). Starch was analyzed in the laboratory by the SPRI modification of the method developed by the Sugar Milling Research Institute (10). Cold tolerance test samples and some maturity test samples were analyzed for those polysaccharides.

Selection of wavelengths.

The vibrational modes of bonds for sucrose molecules were examined, using a saturated solution of sucrose in water, and were found to display unique absorptions at 2088 nm and 2272 nm, with HOH groups absorbing at 1435 nm and 1940 nm. Figure 2 compares this spectrum with those of several cane juices. Combinations of these wavelengths with wavelengths indicated by the software as highly

correlated with components were used for Brix, pol and purity calibrations in cane juice and beet juice.

The absorption bands in the NIR region are quite broad and overlapping, so that it is difficult to determine the optimum spectral bands for weakly absorbing components, such as sucrose. By converting (through software) the spectrum to the second derivative, overlapping weaker absorbers are separated and peak intensities of these separated absorbents are enhanced, providing spectral bands which may be assigned to each absorbing component.

RESULTS AND DISCUSSION

Sugarcane factory analyses

Calibration curves were developed for pol, Brix and purity as follows: cane juice analyses from the Florida and Louisiana locations, and over two crop seasons, were combined to generate calibration curves. A total of 218 samples was used for the calibration: the curve is shown in Figure 3. The calibration curves for Brix and purity, using the same sample set, are shown in Figures 4 and 5.

Calibration statistics are listed in Table 1. Standard deviations on regular lab methods were supplied by the factories which conducted the lab analyses.

All values were included in the calibration curve in this first study: if outlier values were to be discarded, precision and correlation would appear better. Multiple correlation shows the goodness of fit of data to a straight line.

Spectra from a set of Florida samples run later in 1992 was used to predict lab values from these calibration curves. Another set of samples from Louisiana in 1991, was also used to predict lab values, i.e. the NIR spectra were fed into the computer, which analyzed them according to the calibration curve for each component and reported a predicted lab value. Validation results for pol on the Florida set are shown in Figure 6, and all results are summarized in Table 2.

The difference in predicted values between Florida and Louisiana sets may be explained by the difference in lab method sample treatment, since Louisiana lab methods, before pol analysis, use a chemical treatment which can give greater variation in results than the simple membrane filtration used in Florida lab juice analysis. A small positive bias was observed for both pol and Brix on the Louisiana test set, which can also be explained by the difference in sample treatment for the lab values. The reagent used in the

Louisiana juice clarifications has been observed to give an increase in Brix, and decrease in pol; these differences account for the bias observed in the Louisiana validation set.

In general, NIR predicted results compare well with standard methods of juice analysis for pol, Brix and purity.

Initial results on NIR analysis of bagasse for pol and moisture are very promising. Traditional lab methods of oven drying for moisture and blender extraction for pol are so time consuming and cumbersome that only a few samples are run each day. NIR analyses could make possible more frequent analysis, and analyses of bagasse from each mill as well as final bagasse, to show efficiency of extraction at each mill in a short time. Feedback control would then be possible on each mill.

Initial results, on a small set of bagasse samples over a narrow range of component concentration are shown in Table 3. Increase in the number of samples and extension of the range of pol and moisture content will increase the precision of the results which do, however, compare favorably to the standard deviation values of current lab methods.

A repeat analysis (11 times) of one bagasse sample to determine error caused in filling the cell showed that about 0.1% of the calibration error at 51.00% moisture comes from the error in loading the cell, i.e. in sampling the bagasse sample brought to the laboratory.

Results on fiber in whole sugarcane are at this point promising, with multiple correlation coefficient of about 0.8; however, a detailed spectral analysis of cane fiber spectra is being carried out to improve the accuracy of this determination.

Results on the NIR determination of "true" sucrose, by HPLC (as opposed to "pol" sucrose) are very good, as shown for calibration and prediction sets in Table 4. The use of such a calibration means that HPLC analysis of cane juice can be brought down to a 2 minute per sample time scale, and will therefore be a practical analysis for grower samples as well as for factory process control. Once the calibration curve for HPLC sucrose is in the NIR control computer, along with calibration for pol, Brix and purity, the single 2 minute juice analysis serves to generate results for all four analyses: pol, Brix, purity and HPLC sucrose. When invert calibration is improved, this analysis can be added. Again, no sample preparation or chemical treatment is required. The standard error of prediction, shown in Table 4, for sucrose is lower than the standard error of calibration, because the prediction set all fall within the high range of the calibration.

Results for HPLC glucose are not as good as those for sucrose, probably because the range of sample values is so small. Work on the glucose calibration, and on those for fructose (HPLC) and total invert (HPLC) in cane juice is continuing.

Sugarbeet factory analyses

Results presented herein for sugarbeet factory analyses are the first results obtained, on small sample sets of 50 samples of each juice from each factory, and so are preliminary. The two factories participating in this early study represented beets from two different areas, with quality differences in the two areas because of disease and weather. Nevertheless, it was found that calibration curves combining the data from both factories gave satisfactory correlations and standard errors of calibration for both pol and Brix. Purity calibrations are not shown because of differences in laboratory methods of calculation, but purity errors are expected to be the summation of the pol and Brix errors, as for cane juice. Figure 8 shows the calibration for Brix of thin juice in combined samples from factories A and B.

Both diffusion juices and thin juices were run directly, with no sample clarification or pretreatment, in a 1 mm cuvette.

Very preliminary results on NIR analysis of brei for pol and nitrate have shown promise: there is, as expected, good response to pol, and also response to nitrate. Various forms of sample presentation of brei will be compared in the next stage of this project.

The very preliminary results for factory molasses also show promise for development of good calibration curves, as indicated in Figure 9. Development of NIR calibrations for molasses will increase the frequency of possible analyses by at lease one order of magnitude, and allow real time feedback control on crystallizers.

SUMMARY AND CONCLUSIONS

NIR analysis of sugarcane and sugarbeet juice is rapid and simple, with no sample preparation. Up to 8 components can be analyzed in two minutes (actual run time of analysis is 30 seconds) after calibration curves have been made, so that all samples currently coming into cane factories in, for example, Florida or Louisiana, or beet factories in California, can be tested.

No chemical treatment or clarification is required for juice analysis, so that no chemicals disposal is required. Filtration is not needed for juice analysis; whole, unfiltered juice is analyzed in both cane and beet factories. Heavy mud content in juice does

not appear to inhibit NIR analysis. Juice calibration of pol, Brix, purity, sucrose (HPLC) and glucose (HPLC) have been developed.

On-line adaption of the lab NIR analyses described herein is currently in progress, to allow on-line process control in the factory and has in fact, been applied by British Sugar plc (11). Initial results for beet brei and for molasses analyses are very promising.

It appears that "universal calibration curves" can be developed, using data from several areas and seasons. Formerly, with use of filter NIR instruments, it had been thought that each factory would have to develop their own calibrations, but now the use of the scanning instrument has shown that each factory need not do this. General calibration curves, such as those reported here, can be used. A check set (up to 6 points) would be recommended periodically to maintain accuracy and detect bias.

Initial results on NIR analysis of bagasse are very promising and will be further developed in the next cane harvest. NIR analysis of cane fiber in whole sugarcane is at an intermediate stage, with reasonable correlation values.

NIR analyses for whole cane fiber, raw sugar and molasses, invert, dextran and polysaccharides in juice, and components are now being developed at Sugar Processing Research Institute, Inc., to increase the usefulness of this new generation of scanning NIR instruments to the sugar industry.

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(3) Personnel at Okeelanta Corporation, South Bay, Florida.

(4) Personnel at Sugar Cane Growers Cooperative of Florida, Belle Glade, Florida.

(5) Personnel at Holly Sugar Corporation, Hamilton City, CA and Tracy, CA factories

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Table 1. NIR calibration results for cane juice

ANALYTE NAME	STANDARD ERROR OF CALIBRATION (NIR)	STANDARD DEVIATION (LAB METHOD)	MULTIPLE CORRELATION	RANGE OF VALUES
POL	0.185	0.19	-0.99	13.52-18.56
BRIX	0.212	0.10	0.98	16.74-21.71
PURITY	0.786	0.659±0.1	-0.92	79.00-89.00

Table 2. Validation results for cane juice

ANALYTE NAME	STANDARD ERROR OF CALIBRATION (SEC)	STD. ERROR OF PREDICTION (SEP)	MULTIPLE CORRELATION	NUMBER OF SAMPLES
FLORIDA				
POL	0.185	0.23	0.91	106
BRIX	0.212	0.25	0.87	106
LOUISIANA				
POL	0.185	0.37	0.97	80
BRIX	0.212	0.24	0.98	80

Table 3. Preliminary results for NIR bagasse analysis

ANALYTE NAME	STD. ERROR OF CALIBRATION	MULTIPLE CORRELATION	RANGE	NUMBER OF SAMPLES
MOISTURE	0.76	0.81	50.00-53.00	15
POL	0.22	0.64	2.07 - 2.88	15

Table 4. NIR calibration and validation results
          for sucrose and glucose in sugarcane
          juice by HPLC

ANALYTE	STD. ERROR OF CALIBRATION	STD. ERROR OF PREDICTION	MULTIPLE CORRELATION	NUMBER OF SAMPLES
SUCROSE	0.73	0.45	0.94	324
GLUCOSE	0.18	0.29	0.74	327

Table 5. NIR calibration for pol and Brix
          in sugarbeet diffusion juice and
          thin juice

ANALYTE	STD. ERROR OF CALIBRATION	MULTIPLE CORRELATION	STD. DEVIATION (LAB METHOD)	NUMBER OF SAMPLES
DIFFUSION JUICE FACTORY A				
POL	0.24	0.99	0.20	50
BRIX	0.09	0.98	0.10	50
DIFFUSION JUICE FACTORY B				
POL	0.51	0.92	0.20	50
BRIX	0.08	0.96	0.10	50
DIFFUSION JUICE COMBINED				
POL	0.52	0.99	0.20	100
BRIX	0.19	0.99	0.10	100
THIN JUICE FACTORY A				
POL	0.19	0.99	0.20	50
BRIX	0.09	0.99	0.10	50
THIN JUICE FACTORY B				
POL	0.27	0.99	0.20	50
BRIX	0.17	0.97	0.10	50
THIN JUICE COMBINED				
POL	0.75	0.99	0.10	100
BRIX	0.13	0.99	0.10	100
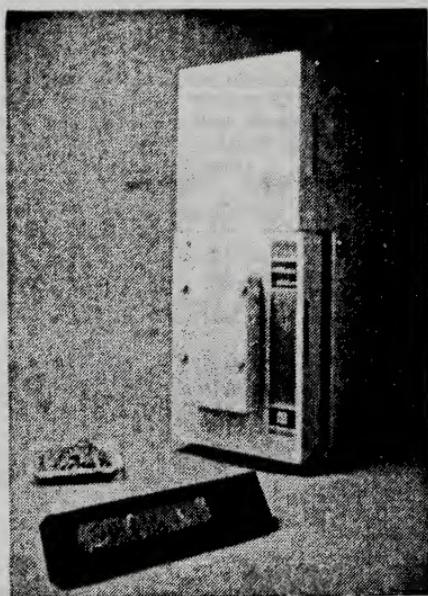
Figure 1. NIRSystems Model 6500 instrument with sample cell for bagasse analysis.
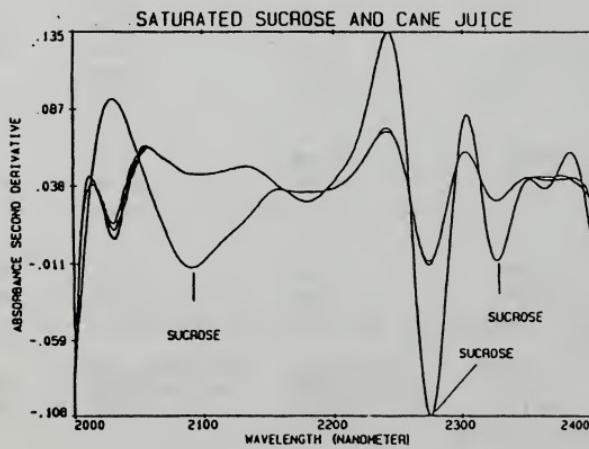
Figure 2. Second derivative curves for saturated sucrose and three cane juices.

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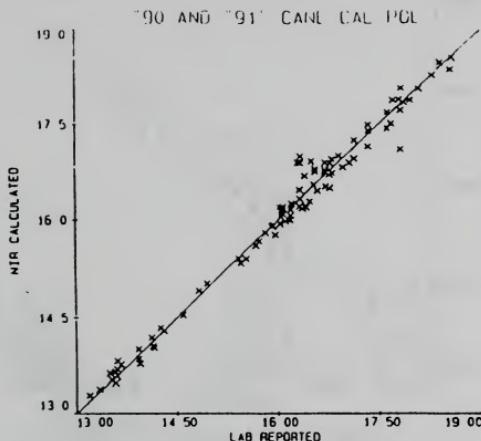
Figure 3. Calibration curve for pol
in sugarcane juice.
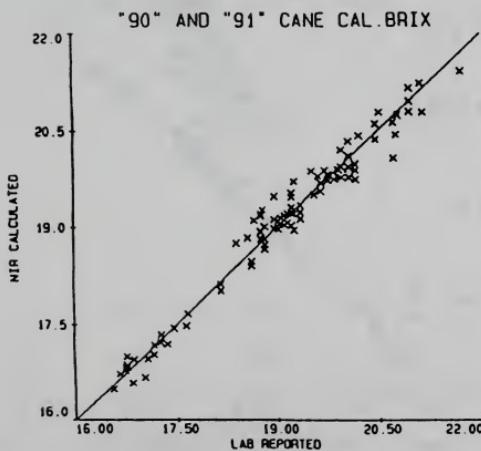
Figure 4. Calibration curve for Brix
in sugarcane juice.
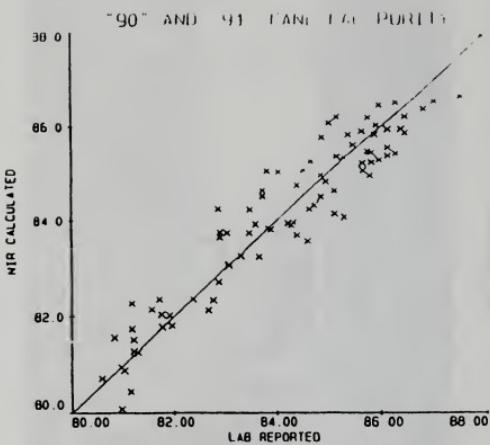
Figure 5. Calibration curve for purity of sugarcane juice.
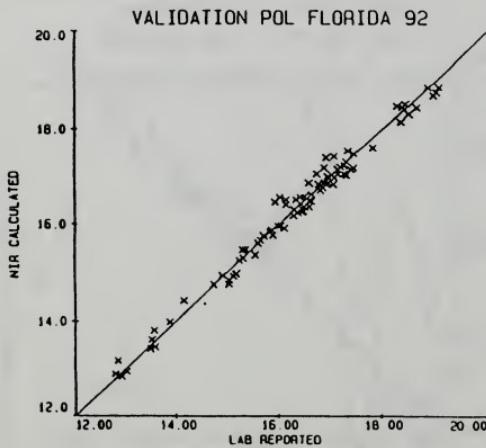
Figure 6. Validation set of samples for pol in Florida sugarcane juice.

NIR CALCULATED VS. LAB REPORTED

CALIBRATION FOR SUCROSE, HPLC
MULTIPLE R = 0.942
STANDARD ERROR = 0.728
NUMBER OF SAMPLES = 324
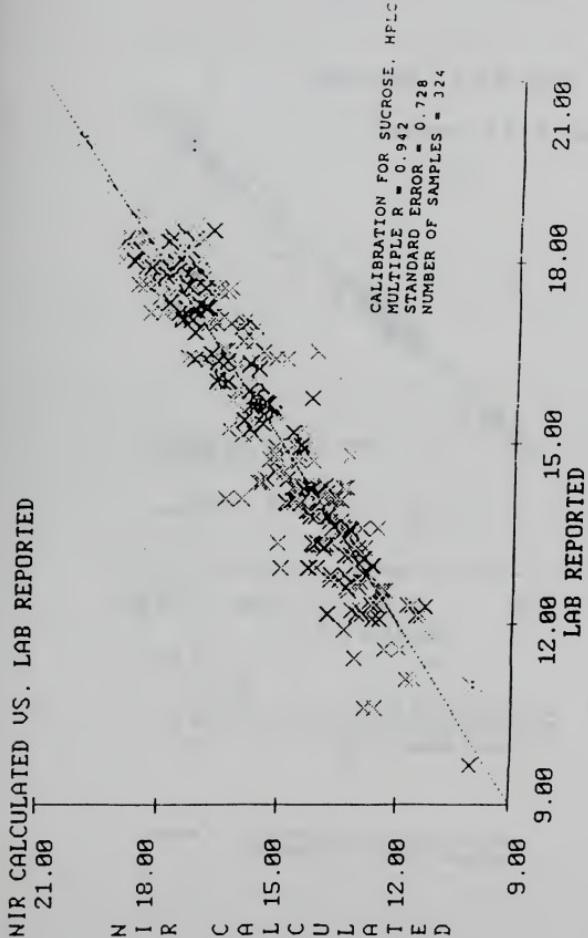
Figure 7. Calibration curve for sucrose
by HPLC in sugarcane
juice.

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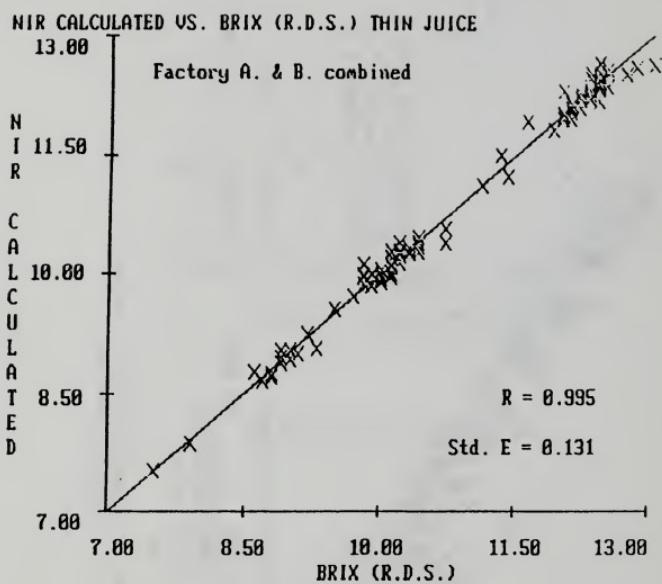
Figure 8. Calibration of Brix in thin juice
from combined samples at
Factories A and B

NIR CALCULATED VS HIGH BRIX IN BEET MOLASSES

R = 0.996
Std. E = 0.163

NIR CALCULATED VS POL IN BEET MOLASSES

R = 0.958
Std. E = 0.171
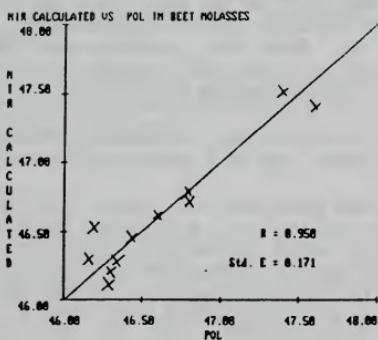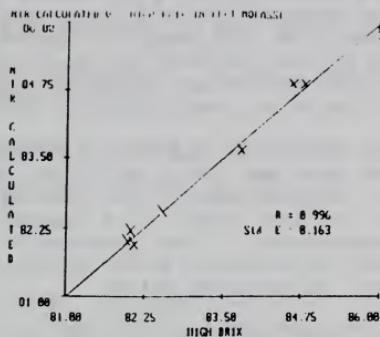
Figure 9.   Initial calibration curve from
            pol and Brix of beet molasses

DISCUSSION

Question: You've shown the possibility of a universal calibration curve, which is what I'm sure everyone would like to see developed. On the purity analysis on the curve for combined areas, the error on purity was bigger because of the combined errors in pol and Brix measurements. If you were to combined the predicted error for NIR for, say, Florida only, is the error significantly different?

Clarke: The error is smaller when only one set of samples are used for the calibration and the same set for prediction, but we have not tested the statistical significance. The purity number is calibrated in the factory, so the error is a summation if the errors in pol and Brix measurement. The prediction error for purity was actually less than the standard error of calibration for purity because of its being a calculated value.

Question: You already have good calibrations for pol and Brix. Why do you make a calibration curve for purity when it can be calculated so readily?

Clarke: Yes – we agree with you – we think purity should be calculated. In the case we used, the factory was used to seeing three numbers (pol, Brix, purity) so we set this up to print out three values. However, the purity value could easily be printed out as a calculated value. Dr. Edye has pointed out that there is little reason to use a physico chemical method to determine a ratio, when it could easily be calculated.

Question: In the pol determination in sugar, does the high concentration of dextran affect the NIR reading for pol?

Clarke: The NIR is calibrated against the laboratory pol reading. If the wavelength chosen for pol is not a sucrose wavelength, then laboratory errors in pol will show up in the calibration for pol. If the laboratory pol reading is affected by dextran, the NIR calibration will be also. If the calibration is for sucrose only, as for HPLC sucrose, dextran will not affect it.

NEAR INFRARED ANALYSIS OF RAW SUGARS AND IN SUGAR REFINERIES

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ABSTRACT

Near infra-red analyses methods for raw sugar analysis are
described. Components analyzed include pol, sucrose, color,
invert, glucose, fructose, total polysaccharides, dextran, starch
and moisture.

An NIRSystems Model 6500 scanning instrument was used
for all analyses. Sample presentations in solid and solution forms
are compared. Scan parameters and chemometric choices are
discussed. Applications for on-line control in sugar refineries
are considered.

Results are presented on the analyses described, and are compared
to currently available methods of analysis for accuracy, precision,
time, personnel and cost.

INTRODUCTION

NIR multicomponent analyses for product evaluation of grain,
cereal, forage and fiber are well documented (15,25). Recently,
NIR spectroscopy has been utilized in quality control of pharmaceu-
ticals (13), and food products, including sugar containing foods
and dry mixtures (17,26). The possibility for application of NIR
analysis to process control in sugar manufacture has prompted
several studies in both the beet and cane sugar industries.

The sugar industry studies include reflectance and transmittance
spectroscopy using both scanning and multiple filter instruments.
Reflectance spectroscopy has been applied to the analysis of whole
shredded sugarcane (20) and whole fibrated sugarcane (1) for pol,
fiber, sugar and moisture. Transmittance spectroscopy has been
applied to the analysis of sugarcane juice (1,6,7,9,15), and
diffusion, thin and thick beet juices (2,3,8,14,22-24) for pol and
refractometric dissolved solids. These NIR analyses of major
components in the sample matrix have generated results with errors

similar to, or slightly greater than, the errors of conventional analytical methods. The advantage of NIR multicomponent analysis over conventional methods is in time and labor savings.

In general, the development of new applications has been more successful with scanning instruments than with multiple filter instruments. Furthermore, advances in chemometric analysis methods have increased both the potential applications and the sensitivity of NIR spectroscopy. With this perspective, we are attempting to broadened the application of NIR spectroscopy in the sugar manufacturing industry to the analysis of minor components in the sample matrix (i.e., glucose and fructose concentrations in cane and beet juices (8,11)).

For example, we have recently described the use of NIR spectroscopy for the analysis of polysaccharides in raw sugar (12). Raw cane sugar contains about 0.1 to 0.2% polysaccharides and about an equivalent amount of high molecular weight (HMW) colorants (>12,000 DA). The source of these compounds and their effects on sugar manufacture are shown in Table 1. In addition to dextran and starch, raw sugars contain other soluble polysaccharides (e.g., cane plant cell wall hemicellulose) that are included in an analysis of total soluble polysaccharides. HMW colorants contribute to the total color of raw sugar and may be chemically associated with some polysaccharides (5).

In the process of refining raw sugar, the quality and yield of the crystalline white sugar product are adversely affected by the presence of these high molecular weight compounds. Regular analysis of incoming raw sugars is an essential element of good process control. However, the conventional methods of analysis, briefly described below, are relatively time consuming. Therefore, replacement of current analyses with a more rapid test would be a significant refinement of the process.

The initiative behind the present study was to replace current analyses that are relatively time consuming and tedious with NIR analyses that are less labor intensive, and potentially on-line and continuous. We targeted analyses in sugar refineries that are performed routinely, and also some that are performed infrequently, or not at all, but may be of interest to refinery management. These analyses include pol, ash, color, invert, glucose, fructose, total soluble polysaccharides, dextran, and starch. Results on all of the aforementioned analyses are reported. The more difficult conventional analyses of the high molecular weight materials in raw sugar (viz., dextran, starch, and total soluble polysaccharides) are discussed in more detail to emphasize the extraordinary advantages of NIR analysis over existing analytical methods.

EXPERIMENTAL

Sixty samples of raw cane sugar were obtained from a world wide selection of raw sugars available at the Sugar Processing Research Institute (SPRI). The samples covered a wide range of quality as well as origin.

Reflectance and transmittance spectra (400 to 2500 nm) were obtained using an NIRSystems 6500 scanning NIR spectrophotometer (Perstorp Analytical Co.). For reflectance spectra, crystalline raw sugar samples were packed into a sample cup with a circular face (5 cm dia., 0.5 cm depth). The spectrum was scanned 50 times and averaged; the cup is mounted on a stepping motor that rotates to present a different orientation of the sample to the light beam for each scan. For transmittance spectra, 50% wt/wt aqueous solutions of the raw sugar samples in a quartz cuvette (1 mm depth) were scanned 50 times and averaged. The raw sugar solutions were not filtered or clarified with chemicals or pretreated in any way.

The spectra were analyzed using NSAS (Ver. 3.22) spectral analysis software (Perstorp Analytical Co.). The software utilizes linear regression and partial least-squares statistical methods to obtain the best NIR wavelengths (or bands) for correlation to data (e.g., for dextran) obtained by conventional methods.

The raw sugar samples were analyzed for dextran (10), starch (4), total polysaccharides (18), pol (19), ash (19), and color (19) using the conventional methods of the sugar manufacturing industry. Glucose and fructose concentrations were determined by ion chromatography (21).

RESULTS AND DISCUSSION

Comparison of conventional methods with NIR analysis

Sixty raw sugar samples of varying quality and origin were analyzed by conventional methods. The Roberts Copper Method (10) for dextran in raw cane sugar, an official method of analysis of the AOAC, is the most complex of the conventional analytical methods; two quantitative precipitation steps are followed by the phenol-H_2SO_4 test. In the method for starch analysis, described in the Cane Sugar Handbook (4), precipitation of total polysaccharides is followed by boiling in $CaCl_2$ to redissolve the starch, the solution is reacted with iodine and absorbance read at 600 nm. For analysis of total polysaccharides, we used a method developed at SPRI (18); precipitation of the polysaccharides is followed by boiling in H_2SO_4 to redissolve the polysaccharides, and the resulting solution is assayed by the phenol-H_2SO_4 test. By comparison to the above mentioned analyses for polysaccharides, the analyses of raw sugar

pol, ash, color, invert, glucose, and fructose are relatively simple. For example, in the analysis of color, a solution of raw sugar is adjusted to pH 7.0, filtered, and absorbance measured at 420 nm.

The conventional methods of analysis for polysaccharides and color in raw sugars, as typically run at a refinery, are compared to a potential on-line NIR analysis in Table 2. The conventional methods of analysis for polysaccharides are time consuming, tedious, and prone to error. The analysis of color and several of the more simple analyses, while not so prone to error, are, nonetheless, often performed infrequently in sugar refineries. By comparison, NIR instruments are potentially capable of simultaneous analysis of pol, ash, color, invert, glucose, fructose, dextran, starch, and total polysaccharides in under 5 minutes. Furthermore, continuous analysis of incoming raw sugars by a on-line NIR instrument would improve process control (especially affination, filtration, clarification and decolorization) and, as a consequence, improve product quality.

NIR analysis of raw sugars and calibration

The reflectance NIR spectra (crystalline raw sugars) and transmittance NIR spectra (50 % wt/wt aqueous solutions) of the 60 raw sugar samples were subjected to a statistical calibration process using NSAS (Ver. 3.22) software (Perstorp Analytical Co.). The 2nd derivatives of the spectra were correlated to analysis data obtained by conventional analytical methods to acquire the best NIR wavelengths or bands from which a calibration equation could be generated. No more than two wavelengths were used in each calibration equation. The wavelengths and calibration equations for NIR analysis are not reported here, as they are, at this point in time, still undergoing optimization.

Examples of "calibration curves" for NIR reflectance and transmittance spectral analysis are shown in Figures 1 to 6. These scatter diagrams show the fit of NIR generated results to analysis data obtained by conventional methods. The correlation coefficients and standard errors of both reflectance and transmittance spectral analysis are shown in Tables 3 to 5, along with the ranges, means, and standard errors of the data generated by conventional methods.

The NIR analysis results for pol, ash, and color are shown in Table 3. The correlation coefficients and standard errors for the transmittance and reflectance analyses are comparable. These NIR analyses, and especially the analysis of raw sugar color using absorbance at one wavelength, correlate well with the conventional methods (the standard error will decrease as the sample size increases). Although the standard errors are too high for NIR analysis to replace the conventional methods for payment purposes,

the rapid and relatively accurate NIR method has definite applica-
tion to on-line analysis of raw sugars and possibly other liquid
streams in the sugar refinery. We have previously reported
preliminary results (9) on NIR reflectance spectroscopic analysis
of pol and color on a separate set of 30 raw sugars. The prelimi-
nary results are essentially similar to results in this report,
and, therefore, suggest that the calibrations have some robustness.

The NIR analysis results for glucose, fructose, and invert are
shown in Table 4. The NSAS statistical software was unable to find
good correlation between the conventional analytical data (ion
chromatography). These rather poor correlations may be related to
either, deterioration of some of the sugar samples in the time
between NIR analysis and ion chromatographic analysis, or errors
inherent in any conventional method of analysis of monosaccharides
in concentrated sucrose solutions.

The NIR analysis data generated from the transmittance spectra were
superior to data generated from reflectance spectra. In raw
sugars, the sucrose crystals are coated with a syrup film that
differs in composition (e.g., color intensity) to the inner sucrose
crystal lattice. Light penetration across a reducing sugar
concentration gradient, from the syrup film into the sucrose
crystal, may be responsible for the poorer correlation of the
reflectance data. By comparison, the raw sugar solutions present
a uniform matrix for measurement of transmittance.

The NIR analysis results for dextran, starch, and total soluble
polysaccharides are shown in Table 5. The correlation coefficients
for the polysaccharide analyses obtained from the reflectance
spectra data are marginally better than those obtained from the
transmittance spectra data. This improved fit is probably due to
a dilution effect (viz., the raw sugars were dissolved in an equal
weight of water before obtaining the transmittance spectra).
Therefore, the polysaccharide concentrations are near to the limit
of detection of the present NIR instrument. Light penetration
across a polysaccharide concentration gradient, from the syrup film
into the sucrose crystal, may be responsible for the high standard
errors in reflectance data.

The NIR analyses of polysaccharides show precision of about 1/2 to
1/3 of the precision of the conventional analysis. However,
results for these analyses can be obtained in less than 5 minutes,
as opposed to up to six hours for the conventional analyses. This
precision may be satisfactory for the purpose of continuous process
control. Therefore, NIR analysis may have application in sugar
refineries as an on-line process alarm to incoming high polysaccha-
ride raw sugars, or to *Leuconostoc sp.* infection producing dextran
in process.

Potential for on-line Analysis

If the NIR analyses discussed in this report (e.g., pol or dextran) are each appraised on individual worth, the critic may conclude that NIR has only limited application to process control in the sugar refinery. It is true that, at this point in development of NIR analysis of raw sugars, the NIR results have lower confidence levels and higher errors than conventional analytical methods. However, the extraordinary advantage of NIR analysis is in continuous multicomponent analysis (i.e., the sum of all the analyses discussed above performed simultaneously and continuously). Therefore, for process control, we envision a holistic approach to the interpretation of NIR analysis data. For example, continuous analysis of incoming raws alerts an operator to the presence of raw sugar with a lower than normal pol (viz., lower than the rest of the cargo). By examining the analysis of ash and invert the operator can determine with some confidence if the low pol reading is a consequence of inversion or high non-sugar concentrations. Conversely, by examining the color and total polysaccharide data, an operator could distinguish between a very pure high pol raw sugar, and a raw sugar containing high levels of dextran. Furthermore, these analyses are performed with a single instrument and with less labor requirement than conventional analytical methods. The most exceptional advantage in application of NIR to sugar refining is that data would be available to the decision maker within 5 minutes, so the process could be immediately adjusted.

The potential benefits described above support continuing research on NIR applications. Other applications for NIR analysis currently under investigation at SPRI include molasses composition and moisture of sugars.

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Table 1. Source of polymers in raw sugar and their effects on sugar manufacture.

COMPOUND	SOURCE	EFFECTS ON SUCROSE MANUFACTURE
Dextran	soil/air microorganisms on injured cane or in juice	- increases viscosity - distorts growth of sugar crystals - lowers yield of products
Starch	indigenous to cane plant	- increases viscosity - lowers yield of products
Other soluble polysaccharides	indigenous to cane plant	- lower yield and quality of products - cause acid beverage floc
HMW colorant	degradation and condensation of sugars and amino acids, and indigenous polyphenolic pigments	- lowers yield and quality of products

Table 2. Comparison of conventional methods with NIR analysis.

ANALYTICAL METHOD	TYPE OF ANALYSIS	TIME TO PERFORM	FREQUENCY IN REFINERY
Dextran[a]	turbidimetric or spectrophotometric	6 hours	1 per 8 hour shift and a daily composite
Starch[a]	iodometric and/or spectrophotometric	6 hours	1 per 8 hour shift and a daily composite
Total soluble polysaccharides[a]	spectrophotometric	2 hours	seldom
ICUMSA color[a]	spectrophotometric	0.5 hours	1 per 8 hour shift
Near-infrared[b]	spectrophotometric	5 min.	potentially on-line and continuous

a Conventional methods of analysis described in references 21-24.
b Simultaneous analysis of pol, ash, color, invert, glucose, fructose, dextran, starch, and total polysaccharides.

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Table 3. Correlation of NIR spectral data with conventional analysis of pol, ash, and color.

CONSTITUENT	CONVENTIONAL ANALYSIS			REFLECTANCE SPECTROSCOPY		TRANSMITTANCE SPECTROSCOPY	
	Range	$\bar{x}$	S_e	r	S_e	r	S_e
Pol[a]	95.08-100.75	99.03	0.15	0.854	0.47	0.814	0.53
Ash[a],[b]	0.04-0.74	0.32	0.01	0.828	0.06	0.889	0.05
Color[b]	335-17710	5380	230	0.954	650	0.985	435

$\bar{x}$ = Mean; S_e = Standard Error; r = Correlation Coefficient
a Units in % wt/wt.
b Units in ICUMSA color units.

Table 4. Correlation of NIR spectral data with conventional analysis of glucose, fructose, and invert.

CONSTITUENT	CONVENTIONAL ANALYSIS			REFLECTANCE SPECTROSCOPY		TRANSMITTANCE SPECTROSCOPY	
	Range	$\bar{x}$	S_e	r	S_e	r	S_e
Glucose[a]	0.01-1.25	0.18	0.01	0.624	0.15	0.736	0.13
Fructose[a]	0.01-1.00	0.19	0.01	0.605	0.15	0.707	0.14
Invert[a]	0.02-2.03	0.37	0.02	0.659	0.28	0.818	0.22

$\bar{x}$ = Mean; S_e = Standard Error; r = Correlation Coefficient
a Units in % wt/wt.

Table 5. Correlation of NIR spectral data with conventional analysis of dextran, starch, and total soluble polysaccharides.

CONSTITUENT	CONVENTIONAL ANALYSIS			REFLECTANCE SPECTROSCOPY		TRANSMITTANCE SPECTROSCOPY	
	Range	$\bar{x}$	S_e	r	S_e	r	S_e
Dextran[a]	40-1550	545	72	0.745	200	0.544	315
Starch[a]	10-605	225	30	0.654	90	0.465	129
Total Polysac.[a]	262-2320	1315	112	0.845	206	0.810	223

$\bar{x}$ = Mean; S_e = Standard Error; r = Correlation Coefficient
a Units in ppm.
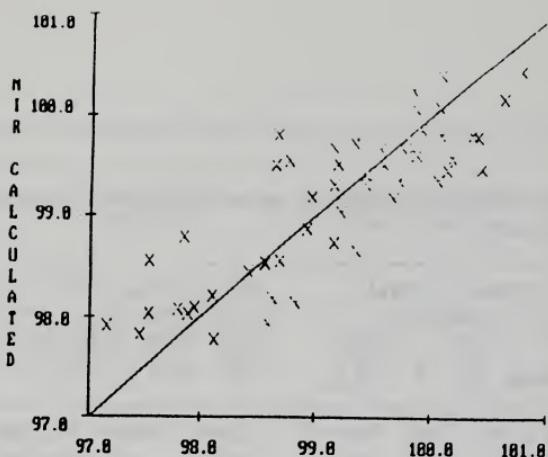
Figure 1. Pol calculated from NIR reflectance spectra versus conventional analysis data.
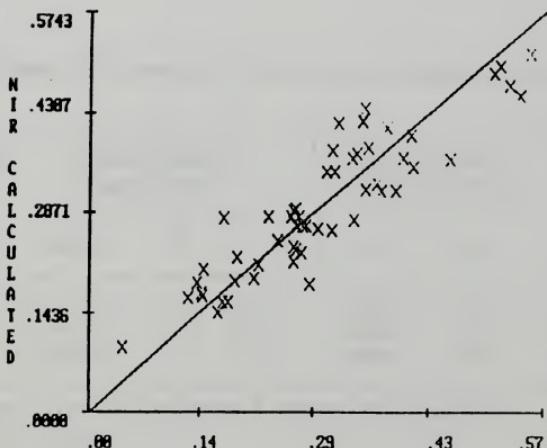
Figure 2. Ash calculated from transmittance spectra versus conventional analysis data.
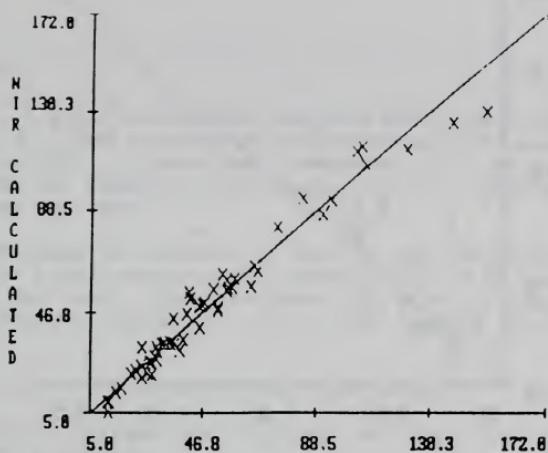
Figure 3. Color calculated from transmittance spectra versus conventional analysis data.
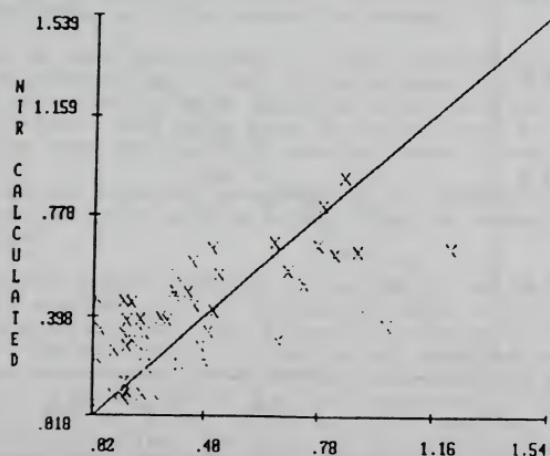
Figure 4. Invert calculated from transmittance spectra versus conventional analysis data.
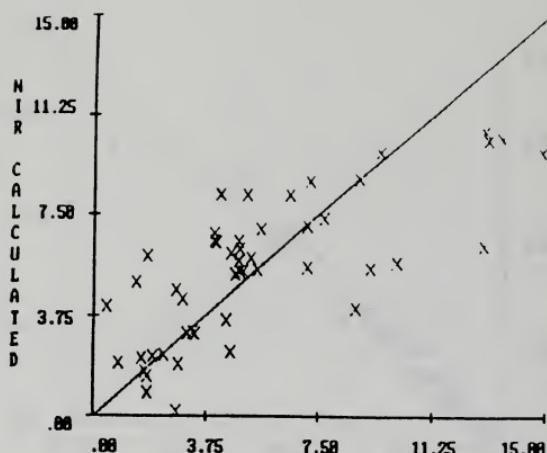
Figure 5.   Dextran calculated from reflectance spectra versus conventional analysis data.
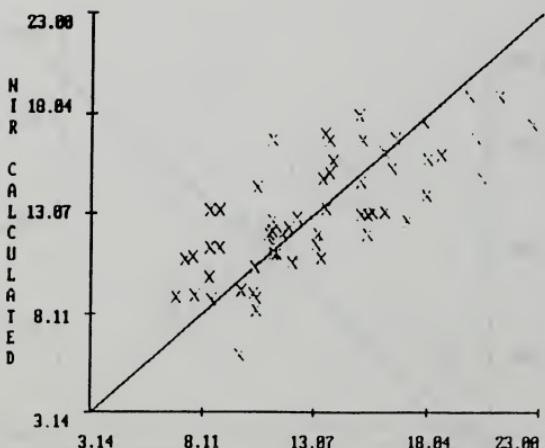
Figure 6.   Total polysaccharide calculated from reflectance spectra versus conventional analysis data.

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DISCUSSION

Question: I want to comment that the SPRI Board of Directors has found that all SPRI sponsoring companies are in favor on continuing research on NIR. I hope that companies will be able to exchange experiences to further this technology so that we are not all reinventing the wheel.

Edye: The issue is whether or not we can exchange data successfully. The requirement will probably be that a scanning spectrophotometer be used. The problems will lie in compatibilities of various types of software. This will require further investigation.

Question: How do you select the wavelengths?

Edye: Let me request that Cynthia McDonald-Lewis of NIRSystems answer that question.

McDonald-Lewis: We first started the investigation of sucrose by scanning saturated sucrose solutions, in transmission mode, and isolating key bands that represented C-H combinations and overtones. For Brix analysis, we look for H-O-H bands from water, because in general there is an inverse relationship between water content and total dissolved solids. As dissolved solids increase, relative intensity of water bands decreases.

Edye: Part of the development of this technology is the demonstration of robustness of the calibrations: will a calibration curve work for a set of data acquired under similar conditions at a different time? The point here is not so much how the calibration works as that the calibration continues to work.

Question: Do you have any observations or comments on wavelengths selected for the true sucrose calibration as compared to the pol determination?

Edye: I would expect the NIR to calibrate better against true sucrose than against pol. I understand this is the case for cane juice calibrations, but I don't have the data yet for this set of raw sugars. The statistical selection of wavelengths for pol were in the areas we expected for sucrose. So, any high dextran sugars could return a larger error for pol compared to the laboratory value. This may be a way of obtaining a reading for pol while excluding dextran effect on pol, if the calibration is made on material that does not contain dextran to influence the pol reading.

Question: A comment or idea: if you store all the spectra in the computer and use principal component analysis, you should be able to locate the best wavelengths.

McDonald-Lewis: We've found that, when you look at principal components, even using partial least squares, the assumptions are not based on any particular wavelength, but on a summation of wavelengths. This does not indicate any particular change in sucrose level per se, but summarizes all the variable changes within the matrices.

COMPARISON OF REFINERY DECOLORIZATION SYSTEMS

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ABSTRACT

Previous studies on refinery decolorization systems in our
laboratory have examined samples from individual refineries and
followed colorant components through process. In this presenta-
tion, we will report on laboratory-scale studies that concentrate
on the efficacy of individual adsorbents to remove colorant compo-
nents.

Four raw sugars were characterized as to 13 color variables,
including, among others, ICUMSA color, phenolics, total poly-
saccharide, color >12,000 daltons, and gel permeation chroma-
tography of 3 critical high molecular weight components. These
sugars were affined in the laboratory, clarified and treated with
6 different adsorbents used in cane sugar refining (new bone char,
regenerated char, new carbon, regenerated carbon, IRA 900 and IRA
958). The effect on the 13 color variables will be presented and
discussed.

INTRODUCTION

Previous studies on refinery decolorization systems in our
laboratory have examined samples from individual refineries and
followed colorant and other components, such as polysaccharides,
through process. While decolorization systems in refineries are,
naturally, optimized for color removal, it is of interest to
examine, as well, the removal of "colorant-related components" by
various adsorbents.   These colorant-related components include
compounds that act as precursors or indicators of color formation,
and such constituents as various polysaccharide types, colorant-
type indicators such as phenolics, amino nitrogen and indicator
value (I.V.), high molecular weight constituents, and ash.

In earlier series of refinery studies, several observations
regarding colorant-related components were made:

Removal of starch: Char, resin and carbon had almost no effect on
starch removal (less than 5% removed) except for almost 62% removed
by a new char filter (7) and 18% removed by service char (Unpub-
lished results).

Removal of polysaccharide: Char alone removed 0%, 30%, and 35.4% of total polysaccharide (TPS) in three different refineries; carbon removed 42%; dual char columns removed 91%; a combination of char and resin removed 94% and resin alone in one refinery removed none (8,9). Another study of 2 refineries (13) showed char removed 40.5% of total polysaccharides at the beginning of a cycle and none by the end of the cycle.

Removal of phenolics: In refinery studies, char removed as little as 27% and as high as 83%, averaging 52% in several refineries; carbon removed from 41% to 78%; a combination of char followed by resin removed 56% and 68%; and a char-carbon admixture removed 44% (4,8,9).

High molecular weight (HMW) color: Char removed 77.3% of total color and 68-75% of HMW color; carbon removed 85% of total color and 80% to 94% of HMW color (9).

MATERIALS AND METHODS

Raw sugars were washed according to a Contract #14 method. Prior to treatment with adsorbent, a 60 Bx washed sugar solution was clarified by filtration through filter aid. Solutions were maintained at 75-80°C during filtration.

Decolorization experiments were done in batch mode using a gyratory incubator set to 75°C. Sugar solutions, at 60 Bx, were treated for one hour; in addition, several char treatments were carried out for four hours. Treatment was carried out with 16.7% adsorbent on sugar solids for char and carbon and 8.3% for resins (Table 5).

Tables 1 and 2 show the pH of the samples. In 11 experiments, pH was adjusted to between 8.3 to 9.0 with NaOH; in 18 of the experiments, the adsorbent alone maintained pH control, which ranged from 7.4 to 9.0. In all char experiments where pH was adjusted, the treatment was carried out for 4 hours instead of 1 hour.

Adsorbents used were new char (from one supplier only), regenerated char (a mixture from several suppliers in use by a refinery), new carbon, regenerated carbon, and Rohm & Haas resins IRA-958 (strong base, acrylic copolymer) and IRA-900 (strong base, polystyrene).

After treatment, resins were removed by filtration on coarse filter paper and char and carbon were removed by filtration on filter aid, maintaining temperature throughout. Filter aid filtration was necessary for the carbon-based adsorbents because the fines were able to pass through the filter paper.

Tests performed included ICUMSA color, HMW color >12,000 daltons (DA), indicator value (I.V.), phenolics, ash, total polysaccharides (TPS), starch, dextran, dialysis for determination of HMW components >12,000 DA, and gel permeation chromatography of HMW components.

Color was measured by the ICUMSA method (13). Ash was determined as conductivity ash, using the ICUMSA method (13). The indicator value was determined as the ratio of color measured at pH 9 and pH 4 (14). Phenolics were determined using the Folin-Ciocalteu reagent (10). Total polysaccharides were measured by the SPRI method (12) and dextran was measured by AOAC method 988.12 (1). Starch was measured by the SMRI method (3).

Dialysis was conducted against flowing deionized water for 100 hours in regenerated cellulose bags with a nominal cut-off of 12,000 DA. The color of the nondialyzable material was used as a measure of the HMW color greater than 12,000 DA, and the dry weight was used to determine the total nondialyzable material (the tenate).

Gel permeation chromatography of the high molecular weight colorant was conducted on 44cm x 2.6cm columns filled with Sephacryl S-500 as described previously (8), with the exception that the eluent used was 0.02M Tris, pH 9.33, instead of water.

RESULTS AND DISCUSSION

Tables A1-A7 in the appendix show the analytical results of adsorbent treatment of the sugars.

Preliminary decolorizing experiments.

Several preliminary decolorizing experiments were done to determine how much adsorbent to use. All treatments were conducted for one hour. Tables 3, 4 and 5 show the results of adding 3.33%, 8.33% and 16.7% of adsorbents on sugar solids. These experiments were conducted on a Nicaraguan lab-washed sugar.

Based on the summarized results in Table 5, it was decided to use the 16.7% level for the char and carbon and 8.33% for the resins.

Decolorization by carbon and resins.

Table 6 shows the results for removal of components by new carbon; Table 7 shows the results for regenerated carbon; Table 8 shows the results for IRA-958; Table 9 shows the results for IRA-900.

Figures 1, 2 and 3, show graphic "profiles" of specific component removal by IRA-900, IRA-958, and new carbon. These profiles highlight the efficient removal of the colorant parameters--color, HMW color and phenolics. They also show the less efficient removal of the polysaccharide parameters--the tenate, TPS, starch and dextran.

Figure 4 compares the removal of TPS by carbon and resins, again showing how difficult this component is to remove.

By comparing the removal of total color to removal of HMW color, as shown in Figure 5, it is possible to note that both carbon and IRA-900 removed less HMW color than total color. IRA-958 removed both to the same degree. This indicates that HMW (>12,000 DA) is more difficult to remove than lower molecular weight colorant. IRA-958 has a greater affinity for HMW color than either carbon or IRA-900.

Char.

The methods employed did not optimize decolorization by char. Extending treatment time for four hours did not improve decolorization; in fact, ICUMSA color and HMW color actually increased in several instances. Chou (11) has shown that batch decolorizing tests using bone char cannot be as efficient as column tests, obtaining an average column decolorization of 73% versus 29% for batch tests for comparable conditions.

Tables 10a and 10b show the results for component removal by new char and regenerated char.

Gel permeation chromatography of HMW colorants.

The removal of high molecular weight colorants was monitored by GPC. Tables 11, 12 and 13 show the percentage removal of several HMW colorants in three sugars. Figures 6-9 show the chromatograms of treated sugars compared to the raw and clarified washed raw. Peaks B, C, and D were combined and treated as a single colorant complex for determining the percentage removed.

Peak A is a polysaccharide-colorant complex with a molecular weight around 2,000,000 DA. It does not contribute very much color, being very pale yellow, but does tend to go into the sugar crystal. The B,C,D peak complex represents the majority of the dark brown color in the HMW colorant. These 3 peaks have an average molecular weight range of approximately 500,000 DA. The HMW colorant makes up about 41% of the total ICUMSA color in raw sugar. The percentage of total color that is HMW color in the GPC samples is listed in Table 14.

GPC results show that each adsorbent had a different affinity for the different HMW colorants. For example, carbon removed relatively more of Peak B and all of Peak D from Florida sugar. Char removed relatively more of Peak C. IRA-900 removed most of Peak C and very little of Peak D. It is interesting to note that IRA-900 produced similar profiles for both the Florida and Australian sugars. IRA-958 removed essentially all of the colorant complex. In another study, we had found that Peak C contains aconitic acid and is more acidic than the other peaks (6). The excellent removal by the strongly basic resins attests to this acidic character.

IRA-958 is a macroreticular, strongly basic resin with a cross-linked acrylic copolymer structure. Its primary advantage is its high color removal and easy regenerability (Rohm and Haas Technical Literature). It is used to remove the high molecular weight dark color bodies and is followed by IRA-900 for polishing to remove final color. IRA-900 is a styrene resin with selectivity and high capacity. The styrene resin (900) is expected to hold the aromatic colorant more effectively than the acrylic resin (958), and is therefore more difficult to regenerate (5).

According to Williams (15) resin removes colorants by 3 mechanisms: ion exchange (based on the charge of the colorant), hydrophobic interaction, and adsorption (based on colorant molecular weight and size and resin pore structure). Colorant with either too high molecular weight or too low charge and too small size will not be removed. The primary mechanism for color removal was reported to be adsorption rather than ion exchange (5). Bento has proposed that this is more true for styrene based resin (2) than acrylic.

In Table 11, besides the removal of Peak A and the colorant complex, the removal of another very high molecular weight peak that is mostly polysaccharide is shown. The data show that this peak is more difficult to remove by any adsorbent. This component has a strong tendency to be included in the white sugar crystal.

CONCLUSION

Table 15 summarizes the mean removal of components for each adsorbent (char is excluded for reasons given above). The following general observations are based on the results of this study:

1. HMW color was more difficult to remove than total color, ranging from 9.0% to 15.7% less HMW removed (mean of 12%) than total color, with the exception of IRA-958, which removed the same proportion of both, thus confirming its reported affinity for high molecular weight colorant. (See Figure 5.)

2. Tenate removal was about the same for all, around 34%. This represents the combined removal of polysaccharides and large colorants. The major portion of the tenate is polysaccharide, which is more difficult to remove.

3. Removal of total polysaccharide did not exceed 35% on average, ranging from 16.0% to 34.6%.

4. Starch was removed less than 20% by the resins and new carbon, but more by the regenerated carbon.

5. Dextran was removed most by IRA-958 (40.1%) and least by IRA-900 (9.7%). Carbon was intermediate (29%).

6. Phenolics were well removed by carbon (70%) and moderately removed by both resins (50%).

7. I.V. was considerably decreased by all except IRA-958, which increased it on most trials.

These results tend to confirm the observations noted in previous studies summarized in the introduction. Ash levels above 700 ppm (0.07%) lower decolorization efficiency of resins (5). In this data, color removal by IRA-958 decreased as ash level increased (correlation, $r=-0.83$); there was also a negative correlation with the initial color ($r=-0.79$). Color removal by IRA-900 showed a negative correlation to initial color ($r=-0.84$), but no correlation with ash content. Carbon was sensitive both to ash ($r=-0.62$) and initial color ($r=-0.96$).

Although char results are not included in these conclusions because results are not yet consistent, it is possible to note that removal of phenolics, total polysaccharides and starch observed in this study are in the same general range as those listed in the introduction. There is a significant drop-off in removal of these constituents from fresh new char to service char.

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Table 1. Final pH of sugar solutions after treatment with adsorbents.

Sugar	Initial	New char	New carb	IRA-900	IRA-958	Regen char	Regen carb
Nic**	6.37	8.72 8.42	7.20 7.38	8.77	7.38	8.17	8.52
FL	5.98	8.16	7.49	8.54 8.48	8.52*	N.D.	N.D.
LA	6.42	8.50*	7.46	8.44	7.39	8.47*	7.44*
Austr.	6.63	8.71 8.44*	8.03	9.00	8.03	8.30	8.39
Brazil	6.09	N.D.	8.24*	8.37 8.89*	7.54	N.D.	N.D.
DR	6.31	8.41 7.80*	7.37 7.76*	9.04	7.41 8.36*	N.D.	8.26*

N.D. = Experiment was not done.
* = pH adjusted prior to treatment (see next table)
** = used for preliminary tests only

Table 2. Listing of experiments where sugar solution pH was adjusted prior to treatment with an adsorbent.

Sugar	Adjusted pH					
	New Char*	New Carbon	IRA-900	IRA-958	Regen char	Regen carbon
FL	--	--	--	9.00	--	--
LA	8.32	--	--	--	8.40	8.59
Austr.	9.00	--	--	--	--	--
Brazil	--	9.00	9.00	--	--	--
DR	8.35	8.59	--	9.00	--	8.30

* All adjusted pH char experiments conducted for 4 hours; all other experiments conducted for 1 hour.

Table 3. Preliminary decolorizing experiments for bone char and carbon

%Adsorbent on solids	% Color removed	
	Carbon	Char
3.33	30.2	7.0
8.33	60.0	15.8
16.7	79.0	27.3

Table 4. Performance of several different chars at 16.7% adsorbent on sugar solids.

Type of char	% Color removed	Observations
New - 1	28.7	
New - 2	35.4	
New - 3	22.3	Used in final studies
Regen - 1	36.7	
Regen - 2	11.9	
Regen - 3	11.5	
Regen - 4	11.3	Used in final studies

Table 5. Summary of decolorization by different rates of adsorbent usage.

Adsorbent	%Color removed	
	8.33% Added	16.7% Added
New char	15.1	22.3
Regen char	9.5	11.3
New carbon	60.5	88.8, 85.2
Regen carbon	63.5	81.1
IRA-958	88.7	91.8
IRA-900	76.3	92.4

Table 6.   Removal of sugar components by new carbon.

Sugar	% Removed of each component						
	Color	Phen-olics	HMW Color	Tenate	TPS	Starch	Dex-tran
LA	84.8	75.9	59.4	27.8	18.1	7.5	2.4
Brazil*	63.4	55.0	46.3	29.8	3.6	2.2	52.7
DR	72.9	76.2	56.3	22.9	13.9	12.8	45.8
DR*	68.0	72.8	45.8	16.8	25.7	12.2	57.9
Austr.	88.7	72.4	81.0	50.0	20.7	29.4	1.9
Florida	89.8	70.7	84.2	55.2	38.7	33.3	13.4

*pH adjusted prior to treatment.   (See Tables 1 and 2.)
TPS = total polysaccharides

Table 7.   Removal of sugar components by regenerated carbon.

Sugar	% Removed of each component						
	Color	Phen-olics	HMW Color	Tenate	TPS	Starch	Dex-tran
LA*	68.0	65.3	68.8	46.1	39.0	63.0	39.2
DR*	74.0	75.5	51.0	11.7	46.7	53.8	43.8
Austr.	88.9	78.3	78.6	69.2	18.1	36.5	5.7

*pH adjusted prior to treatment. (See Tables 1 and 2.)
TPS = total polysaccharides

Table 8.   Removal of sugar components by IRA-958

Sugar	% Removed of each component						
	Color	Phen- olics	HMW Color	Tenate	TPS	Starch	Dex- tran
LA	84.3	56.3	84.4	7.8	45.1	65.0	25.0
Brazil	87.6	43.9	86.3	46.9	9.1	0	54.2
DR*	75.2	48.1	74.0	19.9	20.1	8.3	34.6
DR	80.3	46.6	88.5	40.3	21.4	9.6	31.3
Austr.	88.9	48.4	88.1	54.6	55.6	9.4	52.2
FL*	82.4	52.7	76.9	44.2	28.5	0	43.1

*pH adjusted prior to treatment.   (See Table 1 and 2.)
TPS = total polysaccharides

Table 9.   Removal of sugar components by IRA-900

Sugar	% Removed of each component						
	Color	Phen- olics	HMW Color	Tenate	TPS	Starch	Dex- tran
LA	68.8	61.2	59.4	19.6	16.5	64.3	13.2
Brazil*	50.8	40.8	56.3	44.7	9.1	0	0
Brazil	49.0	43.9	43.8	37.2	0	0	12.8
DR	66.2	48.7	58.3	21.4	2.6	3.4	5.3
Austr.	74.5	52.5	54.8	42.4	10.6	8.2	0
Florida	69.6	56.4	51.2	24.1	46.4	35.9	26.6
Florida	78.0	56.9	70.2	52.2	26.7	19.9	9.7

*pH adjusted prior to treatment.   (See Tables 1 and 2.)
TPS = total polysaccharides

Table 10a. Removal of sugar components by new bone char.

Sugar	% Removed of each component						
	Color	Phen-olics	HMW Color	Tenate	TPS	Starch	Dex-tran
Nic	21.7	32.7	13.5	13.6	35.0	9.0	7.5
Florida	47.0	44.2	50.9	42.2	21.2	29.2	13.8
DR*	41.3	51.7	Inc. +35.4	Inc. +23.3	13.9	39.1	39.6
DR	20.2	29.0	16.7	42.3	13.2	2.1	0
LA*	1.8	39.6	12.5	20.7	34.0	33.3	7.3
Austr.	26.3	37.6	7.9	0	9.1	22.4	27.0
Austr.*	Inc. +11.4	40.3	11.9	34.7	36.3	30.6	13.8

*4-hour treatment; others were 1 hour; also, pH adjusted prior to treatment. (See Tables 1 and 2.)
TPS = total polysaccharides

Table 10b. Removal of components by regenerated bone char.

Sugar	% Removed of each component						
	Color	Phen-olics	HMW Color	Tenate	TPS	Starch	Dex-tran
Austr.	4.3	15.4	Inc. +21.4	11.7	14.6	16.5	46.5
LA*	Inc. +11.8	32.2	Inc. +3.1	21.6	0.6	52.0	49.4

*4-hour treatment; others were 1 hour; also, pH adjusted prior to treatment
TPS = total polysaccharides

Table 11. GPC data for percentage removal of HMW colorants in a Florida sugar. (Refer to Figures 6 and 7.)

Sugar	Peak A A/g*	% Removed	Peaks A,B,C A/g	% Removed	VHMW Polys A/g	% Removed
Raw	723,390	--	8,785,257	--	114,647	--
WRS clar.	578,359	20.0	3,582,258	59.2	102,743	10.4
New carbon	70,494	87.8	720,908	79.9	57,263	44.3
New char	94,888	83.6	1,432,928	60.0	63,887	37.8
IRA-900	117,877	79.6	770,349	78.5	71,303	30.6

*A/g = Chromatographic peak area (A) per gram of sugar; this normalizes all the data so that sugars can be compared.

Table 12. GPC data for percentage removal of HMW colorants in an Australian sugar. (Refer to Figure 8.)

Sugar	Peak A A/g*	% Removed	Peaks A,B,C A/g	% Removed
Raw	513,105	--	9,425,923	--
WRS clar	225,364	56.1	6,186,605	34.4
IRA-900	183,186	18.7	1,110,593	82.0

*A/g = Chromatographic peak area (A) per gram of sugar; this normalizes all the data so that sugars can be compared.

Table 13. GPC data for percentage removal of HMW colorants in a Louisiana sugar. (Refer to Figure 9.)

Sugar	Peak A A/g*	% Remov-ed	Peaks A,B,C A/g	% Re-moved
Raw	1,461,424	--	8,277,011	--
WRS clar.	1,349,190	7.7	4,171,088	49.6
IRA-958	254,485	81.1	26,275	99.4

*A/g = Chromatographic peak area (A) per gram of sugar; this normalizes all the data so that sugars can be compared.

Table 14. Proportion (as a percentage) that HMW colorant is of the total ICUMSA color after various adsorbent treatments (referred to in Figures 6-9).

Sugar/treatment	% of total ICUMSA color caused by HMW colorant
FL clar. WRS	52.6
FL, new carbon	81.8
FL, new char	48.8
Fla, IRA-900	71.4
Austr. raw	40.4
Austr. clar. WRS	41.1
Austr., IRA-900	73.1
LA raw	33.8
LA clar. WRS	30.6
LA, IRA-958	30.5

Table 15.   Summary of mean removal of components by various
adsorbents.

Component	% of Component removed by adsorbent			
	New carbon	Regen car- bon	IRA-958	IRA-900
Color	77.9	77.0	83.1	65.3
Phenolics	70.5	73.0	49.3	51.5
HMW Color	62.2	66.1	83.0	56.3
Tenate	33.8	32.3	35.6	34.5
TPS	20.1	34.6	30.0	16.0
Starch	16.2	51.1	15.4	18.8
Dextran	29.0	29.6	40.1	9.7

Figure 1. SUMMARY OF REMOVAL OF COMPONENTS
IRA-900
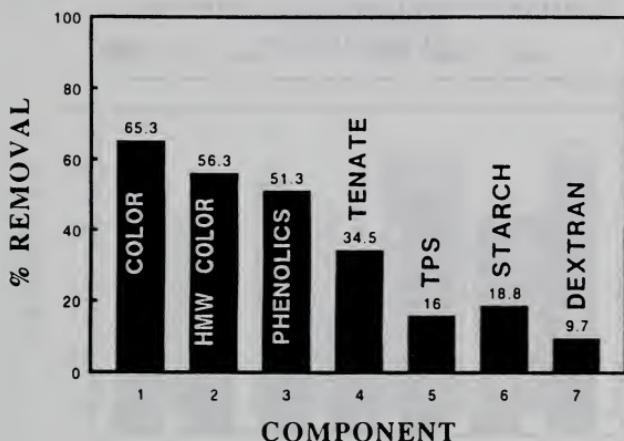
Figure 2. SUMMARY OF REMOVAL OF COMPONENTS
IRA-958
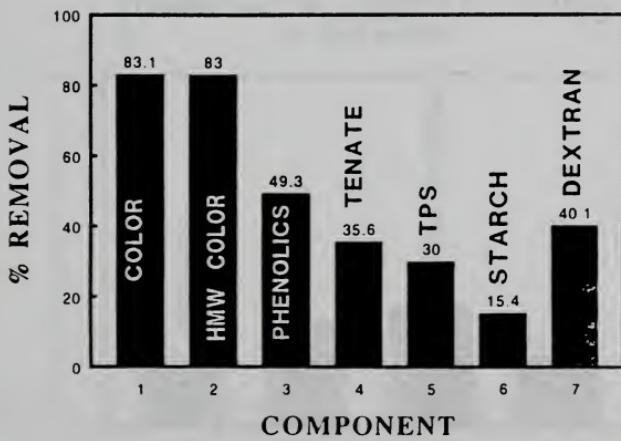
Figure 3. **SUMMARY OF REMOVAL OF COMPONENTS**
NEW CARBON
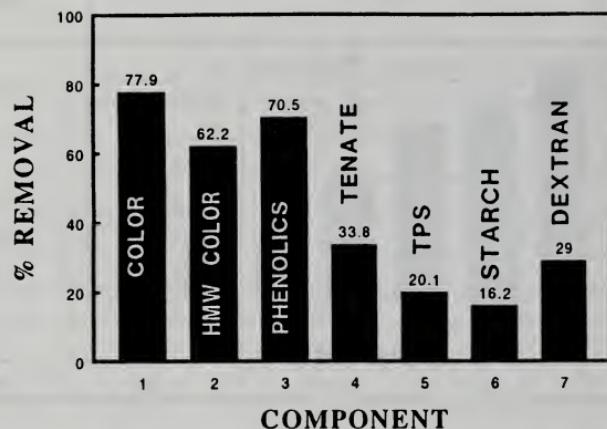
COMPONENT

Figure 4. **SUMMARY OF REMOVAL OF COMPONENTS**
TOTAL POLYS
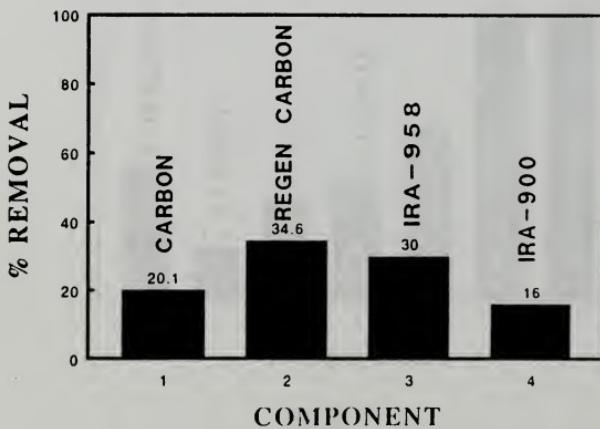
COMPONENT

Figure 5. SUMMARY OF REMOVAL OF COMPONENTS
HMW COLOR
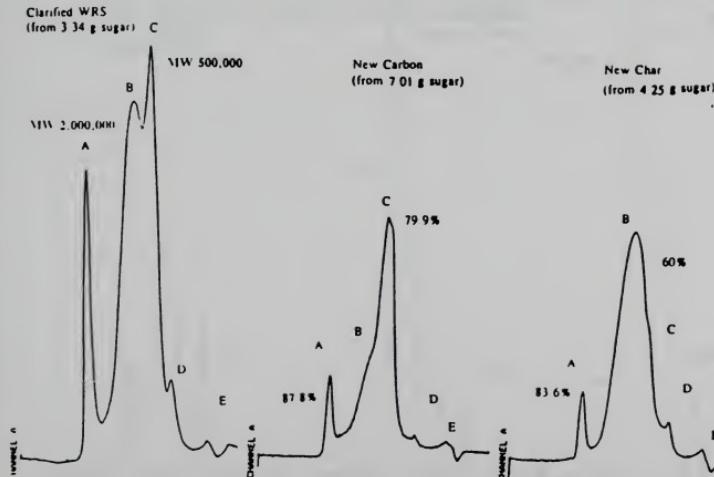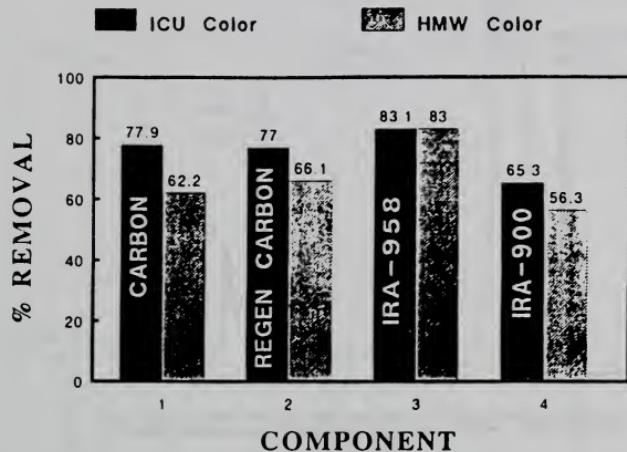
Figure 6. GPC of Florida sugar after treatment with new carbon and new char. The amount of sugar represented by each chromatogram is shown as well as the percentage removal of Peak A and the combined colorant complex (Peaks B,C,D) compared to the clarified WRS.

APPENDIX

Table 1-A. Louisiana Raw Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	New Char*	New Carb	IRA 900	IRA 958	Regen. Char *	Regen. Carb.*
Color	2750	1065	1045	1026	159	326	164	1168	334
I.V.	4.44	4.16	3.88	1.88	2.07	2.81	1.82	2.68	1.74
Phenolics	874	264	245	148	59	95	107	166	85
Ash	0.40	0.11	0.12	0.13	0.12	0.095	0.13	0.11	0.15
TPS	1897	1551	1383	913	1132	1155	759	1375	844
Dextran	675	573	536	497	523	465	402	271	326
Starch	458	299	294	196	272	105	103	141	109
Tenate	2894	1831	1462	1160	1056	1175	1348	1146	788
HMW Color	930	390	320	280	130	130	50	330	100

Table 2-A. Australian Raw Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	New Char	New Carb	IRA 900	IRA 958	Reg Char	Reg Carb	New Char*
Color	2725	1039	1021	752	115	260	113	977	113	1137
I.V.	3.66	3.81	3.41	2.25	1.96	2.13	2.17	2.82	2.30	2.59
Phenolics	512	226	221	138	61	105	114	187	48	132
Ash	0.23	0.09	0.09	0.15	0.09	0.11	0.15	0.10	0.12	0.17
TPS	1125	779	739	672	586	661	328	631	605	471
Dextran	287	163	159	116 ·	156	180	76	85	150	137
Starch	349	96	85	66	60	78	77	71	54	59
Tenate	1904	1044	1021	1038	511	588	464	902	621	667
HMW Color	1100	500	420	390	80	190	50	510	90	370

Table 3-A. Brazilian Raw Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	New Carb*	IRA 900*	IRA 900	IRA 958
Color	3934	1525	1785	. 654	879	911	222
I.V.	2.54	2.77	2.73	1.59	1.97	1.98	3.92
Phenolics	293	263	262	118	155	147	147
Ash	0.32	0.11	0.12	0.13	0.22	0.13	0.16
TPS	1607	1241	1016	979	924	1019	924
Dextran	928	528	546	258	546	476	250
Starch	308	258	135	132	145	158	137
Tenate	2853	1994	1965	1379	1087	1235	1044
HMW Color	2010	730	800	430	350	450	110

Table 4-A. Dominican Republic Sugar -- Results of Treatment with Adsorbents.

Component	Raw	Clar	New Char*	New Carb	IRA 958*	IRA 958	Regen. Carb*	New Carb*
Color	7245	3604	2116	977	894	711	937	1153
I.V.	3.52	2.88	1.75	1.51	3.67	5.35	1.59	1.54
Phenolics	988	534	258	127	277	285	131	145
Ash	0.40	0.18	0.17	0.20	0.36	0.29	0.23	0.23
TPS	2599	912	785	785	729	717	486	678
Dextran	520	240	145	130	157	165	135	101
Starch	454	156	95	136	143	141	72	137
Tenate	3032	913	1126	704	731	545	806	760
HMW Color	2955	960	1300	420	250	110	470	520

Table 5-A. Dominican Republic Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	New Char	IRA 900
Color	7245	1787	1777	1418	601
I.V.	3.52	3.85	3.00	2.22	2.54
Phenolics	988	415	335	238	172
Ash	0.40	0.11	0.12	0.17	0.14
TPS	2599	1236	766	665	740
Dextran	520	194	190	195	180
Starch	454	254	234	229	226
Tenate	3032	2154	1767	1020	1388
HMW Color	2955	1030	960	800	400

Table 6-A. Florida Sugar -- Results of Treatment with Adsorbents.

Component	Raw	Clar	New Char	New Carb	IRA 900
Color	3631	1082	574	110	238
I.V.	2.79	2.78	1.80	1.60	1.90
Phenolics	442	181	101	53	78
Ash	0.36	0.16	0.21	0.16	0.15
TPS	1105	815	642	500	597
Dextran	395	268	231	232	242
Starch	186	171	121	114	137
Tenate	2409	1300	752	582	622
HMW Color	1940	570	280	90	170

Table 7-A. Florida Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	IRA 900	IRA 958*
Color	3631	1282	1245	379	219
I.V.	2.79	2.78	2.61	2.39	3.08
Phenolics	442	181	188	82	89
Ash	0.36	0.16	0.19	0.13	0.27
TPS	1105	922	815	437	583
Dextran	395	326	327	240	186
Starch	186	98	92	59	108
Tenate	2409	1300	838	636	468
HMW Color	1940	570	390	190	90

Table 5-A. Dominican Republic Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	New Char	IRA 900
Color	7245	1787	1777	1418	601
I.V.	3.52	3.85	3.00	2.22	2.54
Phenolics	988	415	335	238	172
Ash	0.40	0.11	0.12	0.17	0.14
TPS	2599	1236	766	665	740
Dextran	520	194	190	195	180
Starch	454	254	234	229	226
Tenate	3032	2154	1767	1020	1388
HMW Color	2955	1030	960	800	400

Table 6-A. Florida Sugar -- Results of Treatment with Adsorbents.

Component	Raw	Clar	New Char	New Carb	IRA 900
Color	3631	1082	574	110	238
I.V.	2.79	2.78	1.80	1.60	1.90
Phenolics	442	181	101	53	78
Ash	0.36	0.16	0.21	0.16	0.15
TPS	1105	815	642	500	597
Dextran	395	268	231	232	242
Starch	186	171	121	114	137
Tenate	2409	1300	752	582	622
HMW Color	1940	570	280	90	170

Table 7-A. Florida Sugar -- Results of Treatment with Adsorbents.

Component	Raw	WRS	Clar	IRA 900	IRA 958*
Color	3631	1282	1245	379	219
I.V.	2.79	2.78	2.61	2.39	3.08
Phenolics	442	181	188	82	89
Ash	0.36	0.16	0.19	0.13	0.27
TPS	1105	922	815	437	583
Dextran	395	326	327	240	186
Starch	186	98	92	59	108
Tenate	2409	1300	838	636	468
HMW Color	1940	570	390	190	90

DECOLORIZATION OF SUGAR PROCESS STREAMS BY MICROORGANISMS

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ABSTRACT

Several fungi have been reported to be effective in
degrading lignins. These microorganisms produce extracellular
peroxidase-type lignin degrading enzymes with broad substrate
specificity. The possibility that these organisms could act on
sugar colorants to make them less colored, or to break down high
molecular weight colorants to lower molecular weight colorants,
provided the basis for this investigation. Lower molecular weight
colorants are less likely to go into the sugar crystal.

The effect of the organisms on color, colorant and composition of
several sugar refinery and factory process and effluent materials
is evaluated. Progress and plans for the project are reported.

INTRODUCTION

The SPRI project on enzymic and microbial decolorization of sugar
processing streams is described in the 1991-92 research plan
approved by the SPRI Research Advisory Committee (Sept. 30, 1991).
This report describes the establishment of a new aspect of this
project (viz. an investigation of the decolorizing potential of
lignin-degrading white rot fungi). This investigation is within
the scope of the SPRI research plan for 1991-92, but is a new
approach to the problems of color formation and removal in the
sugar industry.

White rot fungi derive their name from a class of organisms that
appear in nature as white growths on rotting fallen timber. White
rot fungi produce extracellular peroxidase type lignin degrading
enzymes with broad substrate specificity. Recently, these fungi
and especially *Phanerochaete chrysosporium* have received consider-
able attention from biotechnologists. In addition to degrading
lignin *Phanerochaete chrysosporium* is able to degrade environmental
pollutants (e.g., chlorinated biphenyls, aromatic hydrocarbons,
chlorinated dibenzodioxins), natural and synthetic melanoidins
(i.e., humic acids). Potential biotechnological applications for
these organisms include biological, rather than chlorine-based,
bleaching of paper pulp, and bioremediation of soil contaminated
with the above mentioned environmental pollutants.

In cane sugar manufacture, high molecular weight (HMW) colorant material is difficult to remove from sugar processing streams, and is selectively occluded in sugar crystals. HMW colorant is melanoidin-like, and appears to contain some polysaccharide moiety (in this regard it is somewhat similar to lignin-carbohydrate complexes). Therefore, it seems possible that these fungi may have some ability to degrade HMW colorant.

In the initial stage of this project, we have targeted four white rot fungi (*viz., Tremetes versicolor, Phlebia radiata, Phanero-chaete chrysosporium, Tinctoporellus epimiltinus*) as potential biological decolorants of sugar processing streams. Potential applications include decolorization, breakdown of color precursors, and treatment of waste streams. In addition, the crude extracellu-lar enzyme extracts of these microorganisms may be useful as a tool in the structure elucidation of HMW colorants.

Since this is only a preliminary report, the experimental section (which contains a brief description of experiments rather than details of individual methods) is followed by a section titled "Progress Report" that summarizes the current standing of this research project.

EXPERIMENTAL

Cultures of Tremetes versicolor [TV] (RLG-100), Phlebia radiata [PR] (ATCC 64658), Phanerochaete chrysosporium [PC] (ATCC 24725), and Tinctoporellus epimiltinus [TE] (ATCC 52642) were collected from various sources, and growth tested on potato dextrose agar, Czapek Dox solution agar, and malt extract agar solid media. Clean cultures on malt extract agar solid media were stored for future use.

The four cultures were grown in sterile yeast extract liquid media containing various mixtures of sucrose, glucose and fructose. Phanerochaete chrysosporium was also cultured in sterile mixtures of water and brine wash from an ion exchange column (Ionac 624) of a sponsoring cane sugar refinery and in semi-defined media with 2, 5, and 10 % NaCl added. Growth rates, at 35°C, in shake cultures of the liquid media were determined by either culture dry weight, or simple visual assessment.

Phanerochaete chrysosporium was grown in a 10 liter fermentor (New Brunswick Scientific, Microferm Fermentor) containing 8 L of sterilized media (5g/L yeast extract, 20g/L glucose). The fermentor provided agitation, aeration, and temperature control (at 35°C); pH was not controlled. After 10 days, half of culture

volume was removed and 4 L of unsterilized remelt liquor (ca. 70 Brix, 50 % sucrose; from a sponsoring cane sugar refinery) was added to the remaining fermentation medium at 2 mL/min. The fermentation became infected with bacteria and yeast after about 15 hours.

The culture (4 L, 10 days old) removed from the initial batch fermentation was centrifuged 30 min at 3000 x g and 4°C. The supernatant was subjected to an ammonium sulfate precipitation, followed by dialysis of the precipitate, to obtain a crude extracellular protein preparation. The extracellular enzyme prep. was incubated (at room temperature for 10 hours) with a solution of a raw cane sugar HMW colorant (prepared at SPRI by Earl Roberts). The solution was analyzed at 0 and 10 hours by ion chromatography (Dionex CarboPac PA1) and for color by absorbance (420 nm).

PROGRESS REPORT

Four white rot fungi cultures (viz., PC, TV, PR, and TE) were grown on three solid media types (i.e. potato dextrose agar, Czapek Dox solution agar, and malt extract agar). All cultures preferred the malt extract media; the growth rates of PC and PR were faster than TV and TE on all media.

The growth rate of PC in yeast extract glucose liquid media was measured to determine optimum growth periods for future experiments involving continuous culturing. In a yeast extract solution, containing 2% wt/vol glucose (a shake culture with ca. 10% inoculum, at 35°C), PC remained in log phase growth for 8-10 days. Similar experiments need to be performed for the remaining three cultures.

PC and TV were grown in yeast extract liquid media containing 2% sucrose (Suc), 2% glucose (Glc), and 1% Suc + 1% Glc (shake cultures with ca. 10% inoculum, at 35°C). During the course of these fermentations the sugar remaining in the media was determined by HPLC. Preliminary results indicate that although PC grows faster than TV, the rate of sucrose hydrolysis in the PC culture medium was much slower. In these experiments the yeast extract sugar solutions were autoclaved for sterilization. The resulting sterile media were dark brown due to Maillard reactions. During all fermentations, and particularly with PC, the color intensity of the media decreased. This decoloring activity in semidefined media was considered to be an indication of potential for similar activity with sugar colorants.

The growth of PC in yeast extract glucose at high salt concentra-
tions was followed for 15 days by simple visual assessment.   PC
grew well in semi-defined media with 2, and 5 % added NaCL, but not
at all at 10 % NaCl.   PC was able to grow, albeit slowly, in
undiluted brine wash from an Ionac 624 column (ca. 8.1 % NaCl);
over a 15 day period the pH of this fermentation fell from 7.8 to
5.3, the sucrose concentration decreased from 5.01 % wt/vol to 2.3
% wt/vol and the ICUMSA color decreased from 55828 to 53393.

A large batch culture of PC in yeast extract was prepared in order
to determine if an actively growing PC culture could resist
infection by yeast and bacteria normally present refinery liquors.
Even at the low dilution rate of 2 mL/min of remelt liquor into 4
L of active PC culture, the fermentation soon became infected with
the faster growing yeast and bacteria.

A solution of HMW colorant was incubated with a crude PC extracel-
lular protein extract.   The absorbance (420 nm) of the solution
decreased from 0.282 to 0.267 over a 10 hour period.   The ion
chromatograms of this solution at 0 and 10 hours are shown in
Figure 1.   Although there was little change in the solution color
over the 10 hour period, the chromatograms are significantly
different.   Interpretation of this data is not complete, but it is
tempting to speculate that the enzyme extract changed the molecular
weight distribution of the HMW colorant.

Much work on PC cultures remains to be completed in order to better
understand the activity of this microorganism on HMW colorants.   In
turn, these experiments will be paralleled in the remaining three
cultures.
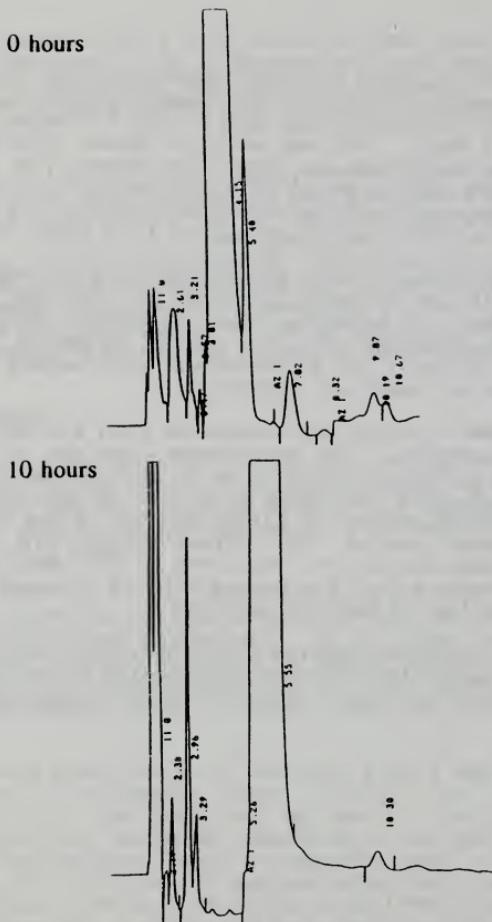
Figure 1. Ion chromatography of high molecular colorant from raw cane sugar after 0 and 10 hours treatment with the crude extracellular enzyme extract from a *Phanerocheate chyrosporium* culture.

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DISCUSSION

Question: A comment: we are working in a similar field at RAR in Portugal, and are also making progress. It's a fascinating new area.

ISOLATION OF A HIGH MOLECULAR WEIGHT COLORANT FROM WHITE BEET SUGAR

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ABSTRACT

A high molecular weight colorant that is preferentially included in
the crystal has been isolated from white beet sugar. This beet
crystal colorant (BCC) has been found to go through a molasses
separation process and has been found in white beet sugars from
several locations in the U.S. and Europe.

Characteristics of BCC include high molecular weight (around 20,000
daltons), faint yellow color, and a positive ninhydrin reaction.
BCC appears to be a polysaccharide, composed of glucose and
arabinose, complexed with $CaSO_4$. Possible mechanisms for its
formation will be discussed as well as observations on a similar
SO_4-complexed colorant isolated from some cane white sugars.

INTRODUCTION

In the course of an investigation at SPRI of the high molecular
weight (HMW) products of a sugarbeet molasses desugarization
process, it was observed that one particular component was
progressively enriched throughout the process, ending up as the
major HMW component in the final white sugar product. A component
with similar properties was observed in several other white beet
sugars, made directly from thick juice rather than from molasses
recovery.   This paper reports our findings to date on this
component, which has been called, for convenience, beet crystal
colorant, or BCC. Brief mention will also be made of a cane sugar
which exhibited a component that was similar in many respects to
BCC.

The two main categories of high molecular weight substances that
impact sugar processing and white sugar quality are polysaccharides
and colorants. They are of particular concern when their structure
and composition favor incorporation into the growing sugar crystal.
Protein, reported to be present at a low level (7-14%) in some of
the polysaccharides isolated during sugarbeet processing (21,22),
is less well characterized.

The polysaccharides of sugarbeet have been a source of concern in processing for many years, as evidenced by the numerous older methods for their analysis cataloged in ICUMSA proceedings and methods book (8-12,17) McCready summarized the polysaccharides of sugar beet pulp in 1966 (14). In recent years, a more intense effort has been made to characterize the polysaccharides of sugarbeet, either from a processing standpoint (4,5,21), or from a nutritional standpoint for utilization of beet pulp as an edible dietary fiber for human consumption (1,7,15,19, 20,23).

Colorants with molecular weights greater than 10,000 DA in beet processing have also been investigated (2,3,6). Until these more recent investigations, greater emphasis had been placed on the colorants with lower molecular weights (13,18), but the larger colorant molecules are proving to be quite interesting because of their tendency to be included in the crystal and to be associated with polysaccharide.

MATERIALS AND METHODS

HMW components were isolated from sugars and syrups by dialysis in regenerated cellulose bags with a nominal molecular cut-off of 12,000 DA. 100 g of sugar solids was dialyzed against running water for 100 hours to remove the sucrose and other smaller molecules. After dialysis, the retained material of MW >12,000 DA (known as the tenate) was concentrated by rotary evaporation at low pressure and freeze-dried.

HMW components in the tenate were chromatographically separated by gel permeation chromatography (GPC) on 2.6 x 44 cm columns packed with Sephacryl S-500 using water at a flow rate of 2.8-3.0 ml/min. Fractions were collected on a Pharmacia Frac-300 fraction collector.

Detection of separation on GPC was accomplished by a refractive index (RI) detector in series with a UV detector set at 214 nm.

Polysaccharide content was determined with a SPRI method (16).

Hydrolysis of isolated component was accomplished using trifluoroacetic acid. Sugars were determined by gas chromatography as their trimethylsilyl derivatives on a DB-5, 30m column.

RESULTS AND DISCUSSION

BCC in a Molasses Desugarization Process.

Table 1 shows the composition of samples from a molasses desugari-
zation process that produces raw and white beet sugar. The process
shows excellent elimination of ICUMSA color and phenolics but less
removal of polysaccharides.

Figure 1 (a-e) shows the GPC pattern (UV-214 detection) of the
samples from the above described molasses desugarization process.
It is clear that the highlighted peak, designated BCC, is succes-
sively enriched by the sucrose separation process and ends up as
the major HMW component in the white sugar. By contrast, the
residual molasses has relatively little BCC compared to the other
remaining colored material, which is absent from the white sugar.
This would indicate that the BCC peak is preferentially included in
sucrose during crystallization. The molecular weight of BCC is
estimated to be around 20,000 DA.

BCC from the white sugar was isolated by GPC for further character-
ization. Among the results are:

1. UV/Vis scan from 200-600 nm showed a strong UV peak with the
maximum possibly at slightly below 200nm. (Our equipment cannot
scan below 200nm.) The addition of alkali caused a slight, over-
all increase in absorbance but no peak shifts or new peaks.

2. A colorimetric test for phenolics was faintly positive.

3. BCC in solution gave a precipitate upon addition of barium
chloride, an indirect test for the presence of the sulfate ion.
Addition of oxalic acid, a test for the presence of calcium ion,
also gave a precipitate.

4. BCC spotted onto a silica gel thin layer plate and sprayed with
sulfuric acid developed a white spot with a brown ring.

5. BCC spotted onto a silica gel thin layer plate and sprayed with
ninhydrin gave a positive reaction, indicating the presence of
alpha-amino acids and therefore, possibly protein. A test of BCC
in solution using bicinchonic acid reagent (another test for
protein) was negative, as well as a Coomassie Blue G-250 test. It
is possible that the solutions were too dilute for these tests,
whereas the material spotted onto a silica gel plate would be
considerably more concentrated and easier to detect. The presence
of a small amount of protein could account for the very large peak

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detected by UV-214 as well as the positive ninhydrin test. A chlorine-toluidine spray, another test for nitrogen containing compounds, was also positive.

6. Analysis of the hydrolysis products by GC showed arabinose and glucose.

7. BCC spotted on a thin layer plate and sprayed with bromphenol blue gave a faint acid response, around pH 4.5.

Because of the formation of precipitates with both barium chloride and oxalic acid, the presence of $CaSO_4$ was suggested, perhaps as a complex with BCC, which may be an arabinogluco-protein. Because it is a small molecule, free $CaSO_4$ could be removed by dialysis. GPC of calcium sulfate showed a different pattern than that obtained from BCC. $CaSO_4$ gave a broad peak detectable with RI at a longer retention time than BCC; also, it did not give the large, sharp peak detected at 214nm, which characterizes BCC.

NMR analysis of isolated BCC is in progress. Future work planned on BCC includes continued isolation of material for elemental analysis and further characterization.

BCC in other beet sugars.

The nondialyzable material (the tenate) of many white beet sugars has a fine granular, free-flowing character, unlike the typical polysaccharide-like character of most tenates (ie, from cane sugar, beet and cane molasses, syrups from both cane and beet processing), which is fluffy, voluminous and cohesive. These beet sugar tenates range in color from light tan to pale yellow to off-white.

A survey of 11 beet sugars from both the United States and Europe showed that 6 had the BCC peak on GPC. All 6 also gave a noticeable precipitate upon the addition of barium chloride and a slight precipitate with oxalic acid. The 5 sugars that did not have BCC also did not react with barium chloride. The somewhat granular texture of the tenates seems to be typical of sugars with BCC.

A set of samples from a different molasses desugarization process had no BCC.

The set of samples in this survey is too small to be able to draw any conclusions about a geographical contribution to BCC -- ie, whether it is associated with high-sulfate soils, sulfitation in process, softening processes, or other factors.

Occurrence of a BCC-like Component in Cane Sugar.

In late 1990, we received a complaint about a raw cane sugar that had produced a very turbid white sugar. We were asked by the refinery, a sponsoring company, to examine the causes for the high turbidity. Table 2 shows the results of chemical tests on the raw and resulting white sugar. Table 3 shows the results of color tests on the white sugar.

The unusually high value of 1644 ppm for the nondialyzable material in the white sugar (this is more in the range of an average washed raw sugar and even some raw sugars), as well as the granular character of the tenate, prompted us to examine this sugar more closely.

Energy dispersive x-ray (EDX) and scanning electron microscopy (SEM) was conducted on the white sugar tenate material. The material showed significant peaks for Ca, S, Si and Cl by EDX.

The reactions of these samples with barium chloride (test for sulfate) were interesting. The whole raw sugar gave a very strong reaction (large amount of precipitate) but the isolated raw sugar tenate did not give any reaction. This would indicate the presence of free sulfate. The reverse occurred with the white sugar: the whole sugar solution gave no reaction with barium chloride, but the tenate showed a copious precipitate. It seemed as if a reaction occurred during processing from the raw to the refined sugar that caused the sulfate to become bound to the polysaccharide.

GPC of the white sugar tenate showed the same chromatographic pattern as BCC in the white beet sugars.

In late 1991, we again received a complaint about a raw cane sugar, from the same mill, that produced a highly turbid white sugar. Again, on testing with barium chloride, we found:

    Whole Raw: Heavy precipitate formed slowly
    Whole White: Negative

    Dialysis results: Raw   Tenate = 3616 ppm
                                Reaction with $BaCl_2$: Negative
                         White Tenate = 1557
                                Reaction with $BaCl_2$: Positive

Filtration of the tenate through a 0.45μ filter did not change the reaction with barium chloride.

In both of these cases the raw sugar came from a mill that used sulfitation in its process.

It is rare to find white cane sugars with a BCC-type peak, but in these two instances, the sugars were associated with high turbidity, a SO_4 reaction only in the tenate, and possible heavy sulfitation in the mill. The fact that no sulfate reaction occurs in the sugar solution could indicate some mechanism whereby sulfate is protected or complexed with the sucrose as well as the polysaccharide and thus not available for reaction with barium. On dialysis, once the sucrose is removed, the sulfate remains complexed to the polysaccharide in the tenate (otherwise it would dialyze out) but it becomes more available to the barium, producing the positive sulfate reaction.

Also unusual for these cane sugars was the extremely high concentration of tenate (more than 1500 ppm) and its appearance as granular and free-flowing, rather than the more typical fluffy, voluminous tenates.

SUMMARY

To summarize, BCC is a high molecular weight component found in some white beet sugars which may be associated with sulfite used in process. It appears to be an arabinoglucan, possibly with some attached protein, and possibly complexed with either $CaSO_4$ or SO_4. It is preferentially included in the crystal during processing and is associated with color development on storage (6).

Further studies are needed to confirm its composition, and more surveys of its presence should be done to determine if it is associated with specific processes or other factors such as high sulfate soils.

A similar, BCC-like component in white cane sugar is associated with sulfitation in the mill and contributes to high turbidity in white sugars.

ACKNOWLEGEMENTS

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Table 1. Composition of samples from molasses desugarization

Sample	ICU Col-or	Tenate (%)	Polys. (ppm)	Phenolics (ppm)
Raw molasses	48,992	1.79	5707	1810
Product fraction	18,027	0.42	344	1022
Residual molasses	70,619	1.56	637	1561
Raw sugar	390	0.32	558	none det.
White sugar	17	0.30	312	none det.

Table 2. Analysis of cane sugars with high turbidity.

Sample	Polys.	Starch	Dextran	Tenate
Raw	1922	633	273	2987
White	395	105	115	1644
White, washed	395	104	not done	not done

Table 3. Results of color tests on white cane sugar with high turbidity.

| Sample	Unfiltered (total) color	ICUMSA col-or (filtered)	Turbidity (as color)	I.V.
Whole	99	22	77	1.70
Washed	88	11	77	2.10
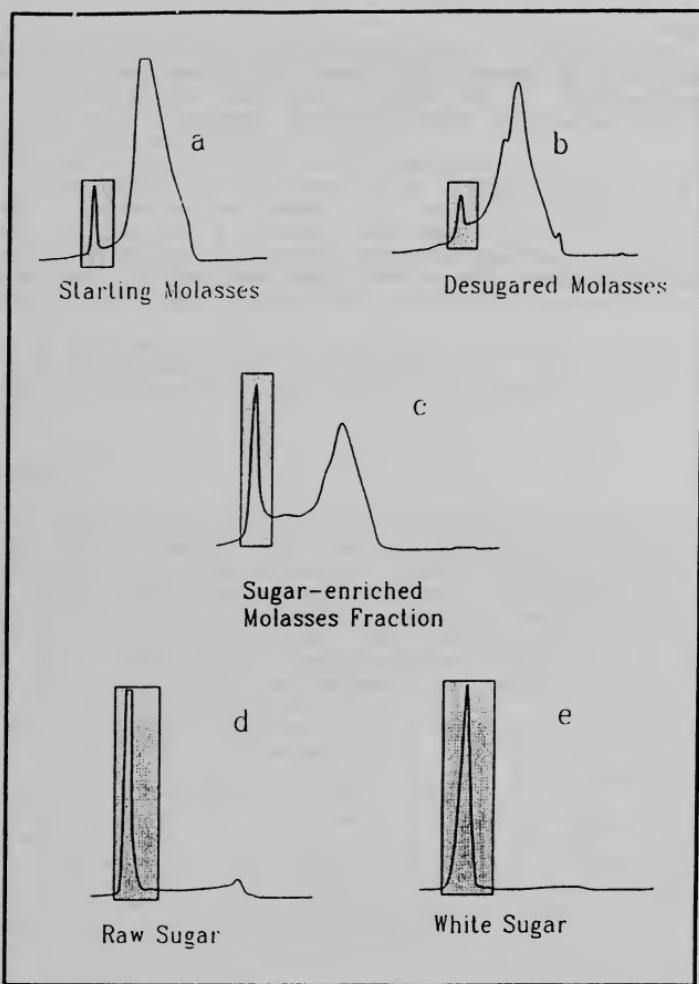
Figure 1. GPC of desugarization process. showing progressive enrichment of BCC through to the white sugar

DISCUSSION

Question: A joint question for this paper, and the previous paper on microorganisms: is the BCC colorant one that might lend itself to removal by treatment with microorganisms?

Godshall: It is a carbohydrate, so that it could be subject to attack by microorganisms. This compound is a light yellow color, imparting a light yellow or buff color to the sugar. One sugar found to contain this compound showed a buildup of color in storage, which could indicate the presence of phenolic acid groups in the color complex. The types of enzymes that Dr. Edye discussed can attach compounds containing phenolic groups.

SUCROSE DECOMPOSITION IN AQUEOUS SOLUTION: A REVIEW WITH
REFERENCE TO PRODUCT LOSS IN SUGAR MANUFACTURE AND REFINING

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ABSTRACT

This review of work on sucrose breakdown and sucrose loss begins
with a survey of sucrose reactions in acid and alkaline media,
including reactions of invert sugar in basic systems.

Reports of studies of sucrose reactions and loss in factories and
refineries are summarized. Conditions that can lead to sucrose
loss, and conditions that can minimize or prevent sucrose loss are
outlined.

INTRODUCTION

The aim of this review is to report on the current understanding of
the mechanisms of sucrose degradation in aqueous acid and alkaline
solutions and to put this knowledge into the perspective of product
loss in sugar manufacture and refining.   Literature on thermal
decomposition and colour formation in aqueous sucrose solutions was
summarized in Sugar Technology Reviews by Kelly and Brown in
1978/79 (28).   They covered topics such as acid and base catalyzed
decomposition of sucrose and hexoses, and included work contained
in 189 references dating from 1932 to 1974.   We do not wish to
repeat such a feat and refer the reader to Kelly and Brown's review
for a thorough historical perspective.   The earlier work of Mauch
(41) on the chemical properties of sucrose, an exhaustive review
containing 272 references, is also worthy of note.   The present
review will concentrate mainly on work reported subsequent to the
period covered by the review of Kelly and Brown (28).   Since 1974,
much has been accomplished, especially in the elucidation of the
mechanism of alkaline degradation of sucrose and hexoses.   Some
commonly held misconceptions on the subject of alkaline degradation
of sucrose will be discussed in detail.

ALKALINE DEGRADATION OF SUCROSE

It is a commonly held belief that the alkaline decomposition of
sucrose proceeds initially via cleavage of the glycosidic linkage
resulting in either D-glucose and D-fructose or ionic forms of
these monosaccharides.   This is in part due to early assertions

that "there is no doubt whatsoever that the influence of alkalis on sucrose consists of a splitting into D-glucose and D-fructose as the first reaction step" (41) and "Alkaline degradation of sucrose to reducing sugars is probably the first step" (59) (in the latter case the authors refer to the review of Whistler and BeMiller (68) on alkaline degradation of polysaccharides which, in fact, does not support such a mechanism). Although these claims were, at the time, intuitive rather than based on evidence, they have been perpetuated by repeated citation, e.g. (13,66,42,63).

In 1970 Parker (50) reported that neither D-glucose nor D-fructose formed even transiently during the hydroxyl-ion catalysed decomposition of sucrose. Parker and others (46) favoured a mechanism wherein an internal nucleophilic displacement at the glycosidic linkage of a sucrose anion resulted initially in ionic forms of the monosaccharides which rapidly formed lower molecular weight acids. The review of Kelly and Brown (28) refers to the work of Parker (50) and notes the "difference of opinion" on the mechanism of base catalysed sucrose decomposition. Although there was some contention, Kelly and Brown favoured an inversion or hydrolysis mechanism.

In contrast to reducing sugars, sucrose and related non-reducing sugars such as alkyl glycosides are considerably more stable in alkali. Several investigators (5,9,24,26,33,43,52) have examined the alkaline degradation of glycosides. In glycosides a *trans* relationship between the aglycon and the 2-hydroxyl group of an aldo-glycoside or the 3-hydroxyl group of a keto-glycoside leads to a much more facile alkaline degradation than is the case for the *cis* analog. Accordingly, α-D-fructofuranosides degrade faster than β-D-fructofuranosides (e.g., the fructose moiety in the sucrose molecule) and β-D-glucopyranosides degrade faster than α-D-glucopyranosides (e.g., the glucose moiety in the sucrose molecule) (*cf.*, methyl β-D-fructofuranoside [1] and methyl α-D-fructofuranoside [2] in Table 1). An internal nucleophilic substitution via the conjugate base (S_NiCB) mechanism has been proposed (43) to account for this effect. This mechanism involves an internal attack at the anomeric carbon by a *trans*-oxyanion (ionized hydroxyl group) resulting in an epoxide intermediate which rapidly hydrolyses and further degrades (see Figure 1). The involvement of an oxyanion α to the anomeric carbon is supported by the relative alkali stability of some O-substituted glycosides (*e.g.*, methyl 3,4-di-O-methyl-α-D-fructofuranoside [3], see Table 1).

In the case of sucrose the ring C-OH groups α to the anomeric carbons on the glucose and fructose moieties are both cis to the glycosidic bonds. Thus, sucrose should be a relatively alkali-

stable glycoside. However, by comparison to related alkyl glycosides (e.g., methyl α-D-glucopyranoside [4] and methyl β-D-fructofuranoside [2]), sucrose is relatively labile to alkaline degradation (see Table 1).

	R_1	R_2	R_3	R_4
[1]	OCH_3	CH_2OH	H	H
[2]	CH_2OH	OCH_3	H	H
[3]	CH_2OH	OCH_3	CH_3	CH_3
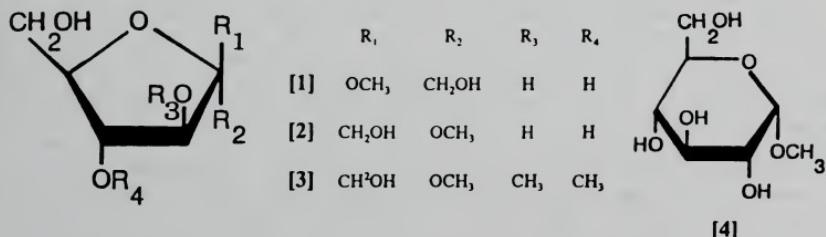
A series of articles (37,38,47,48,56) by Richards et al. on the alkaline degradation of sucrose and some sucrose-like model compounds culminates in a novel hypothesis to explain this alkali-lability. In a study (38) of the alkaline degradation of a mixture of mono-O-methylsucroses, some of the monomethyl derivatives degraded at a rate similar to sucrose, but the 1'- [5] and 3'-O-methylsucrose [6] were degraded much more slowly (primed numbers refer to the D-fructose moiety, as in the numbering system of Hockett and Zief (27)). Earlier work (48) by the same authors had indicated that the mechanism involved the oxyanions of free hydroxyl groups; based on the alkali stability of octa-O-methyl-sucrose [7] and several partially methylated sucroses it was unlikely that the mechanism involved direct attack by hydroxyl ion on the glycosidic bonds. Since only the 1'- [5] and 3'-O-methyl-sucroses [6] survived alkali conditions that would completely degrade sucrose, it was concluded that the oxyanions from 1'-OH and 3'-OH were necessary for the alkaline degradation of sucrose. Simple displacement of the glycosidic linkage by the C-1' or C-3' oxyanion to form a glucose anion and a 1,2- or 2,3-fructose epoxide could be disregarded based on the alkali stability of methyl β-D-fructofuranoside [1] and methyl 3,4-O-dimethyl-α-D-fructofuranoside [3] (methyl α-D-fructofuranoside [2] is alkali-labile [see Table 1] and would react via the $S_N$icB mechanism described above).
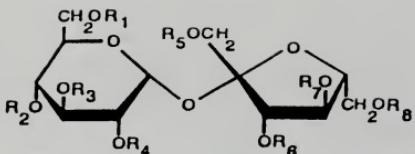
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
sucrose		H	H	H	H	H	H	H	H
1'-O-methylsucrose	[5]	H	H	H	H	CH$_3$	H	H	H
3'-O-methylsucrose	[6]	H	H	H	H	H	CH$_3$	H	H
octa-O-methylsucrose	[7]	CH$_3$	CH$_3$	CH$_3$	CH$_3$	CH$_3$	CH$_3$	CH$_3$	CH$_3$

Richards et al. (38,47) proposed that the reaction proceeded *via* an $S_N$icB mechanism wherein the substitution at the C-1 of the D-glucose moiety by oxyanions derived from 1'-OH or 3'-OH resulted in 1- or 3-O-β-D-glucopyranosyl-D-fructose (see Figure 3). The mechanism implies that the 1'-O-methylsucrose is degraded via 3'-displacement and the 3'-O-methylsucrose via the 1'-displacement.

The relatively slow internal substitution reactions are followed by alkaline degradation of the 1- or 3-O-β-D-glucopyranosyl-D-fructose intermediates by mechanisms and at rates comparable to reducing sugars. A major feature of this mechanism is that the alkali catalysed degradation of sucrose does not initially proceed *via* hydrolysis of the glycosidic linkage and the formation of D-glucose and D-fructose.

Sucrose can, however, degrade to D-glucose and D-fructose in slightly alkaline solution at pH up to ca. 8.3 (sucrose is most stable at pH 8.3-8.5 (50,66)), but this degradation proceeds by the normal acid hydrolysis mechanism. In sucrose manufacture, therefore, the main reaction causing sucrose loss, between pH 7 and ca. 8.3, is the same acid hydrolysis that occurs at lower or acid pH.

ALKALINE DEGRADATION OF MONOSACCHARIDES

D-Glucose and D-fructose are present in both cane and beet juice and decompose on the addition of lime at high temperatures during juice purification. During juice purification and concentration it is necessary to keep invert (i.e., D-glucose and D-fructose) concentrations low. In addition to direct sucrose loss to invert, undesirable dark coloured products may form in the juice via the Maillard reaction of amino acids and reducing sugars. In general, beet juice contains less invert but more nitrogen-containing compounds than cane juice, and while deliberate and complete destruction of invert during juice purification is desirable in beet sugar manufacture, invert destruction is less critical to good process control in the cane sugar mill. In fact it is considered an advantage, in cane sugar manufacture, to maintain a small amount of invert throughout the process, as invert, which remains in the mother liquor during crystallization, has a net effect of lowering sucrose levels in final molasses. Therefore, although alkaline degradation of sucrose does not initially proceed via monosaccharide intermediates, alkaline transformations of monosaccharides are still of importance in sucrose manufacture.

A recent dissertation (17) by de Bruijn, "Monosaccharides in alkaline medium: isomerization, degradation and oligomerization" and other recent publications by de Bruijn et al. (16,18,19,20) well represent the state of knowledge of the complex network of reactions of reducing sugars in aqueous alkaline solution. This complex network of reactions involves both reversible and irreversible reactions (see Figure 3).

The reversible reactions are initiated by an equilibrium between neutral and ionized forms of the monosaccharides (see Figure 4). The oxyanion at the anomeric carbon weakens the ring C-O bond and allows mutarotation and isomerization via an enediol intermediate. It is this reaction that is responsible for the sometimes reported occurrence of D-mannose in alkaline mixtures of sucrose and invert; the three reducing sugars are in equilibrium via the enediol intermediate. The mechanism of isomerization, known as the Lobry de Bruyn-Alberda van Ekenstein rearrangement, generates the enediol anion intermediate that may undergo nonreversible degradation reactions.

The first step in the nonreversible degradation reactions is the formation of a reactive α-dicarbonyl species through β-elimination of hydroxide. The subsequent reaction pathways to all degradation products can be described by just five reaction types, viz., β-elimination, benzilic acid rearrangement, α-dicarbonyl cleavage, aldolization, and retro-aldolization (see *Figure 5*) (23). Retro-

aldolization and α-dicarbonyl cleavage involve C-C bond scission and result in lower molecular weight products. Benzilic acid rearrangement essentially involves addition of hydroxide, and β-elimination, the abstraction of hydroxide. In the aldolization reaction an α-dicarbonyl compound reacts with another carbonyl to form an α,β-dihydroxy ketone or aldehyde with consequent C-C bond formation increasing C-chain length. A somewhat simplified reaction scheme is shown in Figure 6 (22).

De Bruijn et al. (16-20) used modern chromatographic and spectro-scopic techniques to analyze the effect of reaction variables (e.g., pH, and monosaccharide concentration) on the product profile, and developed a reaction model (see Figure 7) that emphasized the role of α-dicarbonyl compounds.

Some of the features of the model shown in Figure 7 are;

(1)   high HO^- concentrations and the presence of Ca^{2+} rather than monovalent cations favour route 2 over 4a (i.e., lactic acid formation is favoured by high pH),

(2)   in the formation of ≤C6 acids (i.e., organic acids containing 6 or less carbon atoms), at HO^- concentrations greater than 10 mM, route 5 occurs preferentially to route 6 and lactic acid and saccharinic acids predominate in the product,

(3)   the precursors for >C6 acids (i.e., >C6 α-dicarbonyls) are assumed to form by aldolization of α-dicarbonyls with other carbonyls (route 7), and termination of this oligomerization is via route 5 to a >C6 acid or route 6 to an aldehyde a >C6 acid or a ≤C6 acid (depending on the size of the α-dicarbonyl),

(4)   moderate HO^- concentrations (1 to 10 mM) and high mono-saccharide concentrations (>10 mM) form >C6 acids in yields up to 50% based on monosaccharide.

The >C6 acids have molecular weights equivalent to 2 to 4 monosac-charide units, and there appeared to be a direct relationship between colour formation and >C6 acid formation. The reaction pathways to >C6 acids provide an explanation for a phenomenon often observed in sugar refineries during carbonatation and evaporation, viz., the disappearance of invert with concurrent appearance of colour (14,62). The temporarily high pH (up to pH 10.5) in refinery carbonatation initiates the formation of higher molecular

weight acids via cross-aldolization of α-dicarbonyls, (16-20) and further degradation can proceed during subsequent filtration, and evaporation.

ACID HYDROLYSIS OF SUCROSE

The investigation of the reaction of sucrose in aqueous acid solution has a long history; it was the subject of several kinetic studies in the early 19th century (15,64,69) and Arrhenius (4) developed the equation describing the effect of temperature on reaction rate using data from sucrose hydrolysis experiments. At the time of the review of Kelly and Brown (28) it was generally accepted that the acid-catalysed sucrose hydrolysis mechanism involved protonation of the glycosidic oxygen followed by heterolysis of the glycosidic bond to form the two monosaccharides, with one monosaccharide in the form of a cyclic carbonium ion (see Figure 8). Other mechanisms, for example via protonation of a ring oxygen atom (8), had been proposed but held little favour. BeMiller (7) preferred fructosyl-oxygen cleavage over glucosyl-oxygen cleavage based on relative hydrolysis rates of fructofuranosides and glucopyranosides and the stability of the sp^2-hybridized carbonium ion in 5- and 6-membered rings. Richards (57) also favoured fructosyl-oxygen bond cleavage and used this mechanism to explain the increase in rate of sucrose inversion in the presence of divalent cations (see section below on inversion rates and sucrose loss). However, the site of bond cleavage remained to be confirmed.

In 1988 Mega and Van Etten (45) reported the use of ^{18}O shift in ^{13}C NMR to elucidate the point of bond cleavage in the acid-catalysed hydrolysis of sucrose. Sucrose was hydrolysed in the presence of $H_2^{18}O$, and the incorporation of ^{18}O into the products was determined. The results clearly indicated fructosyl-oxygen bond cleavage.

Therefore, acid-catalysed hydrolysis of sucrose initially yields D-glucose and a fructose carbonium ion which can react with water to form D-fructose and regenerate H^+ catalyst. As a consequence, further acid degradation of sucrose can be described by the action of acids on D-glucose and D-fructose.

ACID DEGRADATION OF MONOSACCHARIDES

The acid-catalysed reactions of reducing sugars are complex, and in many ways, at least initially, similar to the reactions in alkali (53). Under mild acid conditions (viz., pH 5-6 at 0-60°C) reducing

sugars ionize and mutarotate, at lower pH (viz., down to pH 3 or 4) and at higher temperatures (viz., up to ca. 100°C) enolization and isomerization occurs.   In alkaline solution enolization is initiated by base attack and abstraction of a proton α to the carbonyl group (see Figure 4).   In acid solution enolization is initiated by direct protonation of the carbonyl group (see Figure 9).   In fact, acids are far less effective enolization catalysts than alkalies and as a consequence D-glucose and D-fructose in aqueous solution show maximum stability between pH 3 and 4 (40,44).   It is clearly indicated in Kelly and Brown's review (28) that, under conditions described above, the further acid-catalysed reactions of reducing sugars (e.g., dehydration to 5-(hydroxy-methyl)-2-furaldehyde [HMF]) are extremely slow. For example (29), while sucrose (2.0 M) hydrolysis at pH 5.6 and 100°C was measurable in a few hours, the further decomposition of the invert was demonstrated in a time scale of over 200 hours.   Similarly, an 80 % wt/wt solution of D-fructose after refluxing for 16 hours at pH 6.9 yielded only 0.1 % D-glucose and 0.6 % HMF (70).   In contrast, a good yield of HMF (20 % of theoretical) is obtained from D-fructose solution in greater than 0.25 M HCl (pH ~ 0) at 95°C in a few hours (70).   At much higher temperatures (e.g., 390°C) and under pressure, in less concentrated acid, high yields of HMF form from sucrose and D-fructose in seconds (32).   These studies were conducted in quite pure sugar solutions, whereas in factory operations, the sugar solutions are relatively complex mixtures of sugars, and other organic and inorganic compounds.

The mechanism of HMF formation from D-fructose and sucrose was reviewed by Antal et al. (3).   Several arguments were advanced for favouring a mechanism involving furanose rings and a fructose carbonium ion over an open chain β-elimination mechanism that proceeds via an enediol intermediate to a 3-deoxyhexulose (a similar mechanism to alkaline degradation).   Several reviewers (2,25,53) including Kelly and Brown (28) (who favour the latter scheme) discuss these alternative mechanisms.   Here it is suffi-cient to note that acid-catalysed degradation of reducing sugars proceeds in a complex reaction network to products of isomeriza-tion, dehydration, fragmentation, and condensation.   A typical product profile for D-fructose decomposition is shown in Table 2.

It should be noted that with the possible exception of isomeriza-tion, none of the above acid degradation reactions would be likely to occur to any extent under conditions found in the sucrose manufacturing industry.   Under acid conditions in raw cane sugar manufacture, most reducing sugar degradation proceeds via Maillard reactions with amino acids, as outlined in Figure 10.   These reactions contribute to sucrose loss and undesirable colour formation.   Although the reactions of amino acids with reducing

sugars are important to the sucrose manufacturing industry they are beyond the scope of this review; instead the reader is referred to more complete treatments of this subject (35,36).

In addition to the above reactions of reducing sugars in acid solution, fructose has been reported to form stable anhydride dimers in strong acid or in the presence of strong acid ion-exchange resins. Thus, at some point after complete hydrolysis of sucrose in the presence of a strong cation exchange resin at ca. 40°C, 100g of product carbohydrate contained 50g of glucose, 44g fructose and 4g of difructose dianhydrides (55). Obviously the rate of formation of these dianhydrides is slow by comparison to the acid hydrolysis of sucrose and it is doubtful that these dianhydrides might form at pH above ca. 6.5. Therefore they are of little significance to sucrose loss in the sugar industry. However, they may be of importance in the manufacture of carbonated soft drinks, where pH is about 3.

EFFECTS OF DEGRADATION REACTIONS ON SUCROSE MANUFACTURE

The preceding sections of this review report on the current understanding of the mechanisms of sucrose degradation in aqueous acid and alkaline solution. Although our knowledge of these reactions has clearly advanced since 1974, the sucrose manufacture and refining industry is essentially faced with the same problems of product loss. Unfortunately, the application of this knowledge to problem solving is complicated by the fact that many of the above mentioned studies were performed at pH, temperatures and sucrose concentrations outside the ranges typically encountered in the industrial situation. However, the following generalizations hold true;

(1)   sucrose degrades in acid far more easily than in alkali, while reducing sugars (the product of acid hydrolysis) are far more reactive in alkali than in acid,

(2)   in acid the rate of hydrolysis of sucrose is faster than the rate of degradation of its inversion products,

(3)   in alkali the rate of degradation of sucrose is much less than the rate of degradation of D-glucose or D-fructose,

(4)   since alkali degradation of sucrose does not result in inversion products, in slightly alkaline solution (pH < ca. 8.5) the loss of sucrose to invert (glucose + fructose) is a consequence of the acid hydrolysis mechanism, which provides D-glucose and D-fructose for further alkaline degradation.

Point (1) is especially relevant to sugar refineries, where pH at
the first part of the process (melter, clarifier) is often low
enough to permit formation of invert, whereas pH later in the
process (decolourization) is high enough to cause degradation of
this invert.   Point (1) is also relevant to sugarbeet factories
(i.e., when sucrose hydrolysis during extraction is followed by
alkaline degradation of invert during juice purification).   Point
(2) relates to the sugarcane factory situation, where pH remains
below 7 for almost the entire process.   Point (3) describes the pH
range in the sugarbeet factory after initial extraction of raw
juice.   In point (4) emphasis is placed on the danger of forming
too much invert during extraction in diffusion since subsequent
destruction of invert causes high overall loss of sucrose and
decreased recovery.

Inversion rates and sucrose loss

The literature over many years has tabulated rates for sucrose
hydrolysis and invert (glucose + fructose) formation, at various
concentrations, pH's and temperatures (50,60,61).   These studies,
in general, were conducted in solutions of sucrose only, with pH
adjusted by addition of acid or base, yielding solutions of very
low ionic strength, inorganic content or ash content.   The much-
quoted tables of King and Jison (61) result from measurement of
hydrolysis rates in 0.5% to 2.5% wt/vol solutions of sucrose; these
tables are reproduced in both the Cane Sugar Handbook (61) and the
Handbook of Sugars (49), in the former case with the unfortunate
accompanying indication that the same rates could be applied in the
65 to 70 Brix range.

Part of the foundation for the above-mentioned extrapolation of
hydrolysis rate data from low sucrose concentration to refinery
liquors at high sucrose concentrations is the assumption that
impurities have no effect on reaction rate.   Parker (50) observed
that increasing ionic strength up to 2M as KCl or 1M as $MgCl_2$
increased the rate of sucrose hydrolysis, but he claimed that "the
effect of salts on reaction rate was not sufficiently pronounced to
be considered significant".   Parker neglected the "marginal
influence of salt concentration" in developing an equation that
related the first order rate constant to pH, temperature, and the
concentrations of water and of sucrose.   In contrast, Clarke (12),
in early studies on the use of HPLC to measure sucrose hydrolysis
rates, observed hydrolysis rates greater than those previously
reported in the literature (49,50,60,61), when reactions were run
at 0.1M KCl (ca. 0.7% KCl).   After 6 hours, at pH7, 90°C, and 60
Brix sucrose in water, 98.62% of the initial sucrose remained,

whereas in 0.1M KCl, 95.15% sucrose remained (a ca. 3.5 fold increase in hydrolysis rate at 0.1M KCl).

More recently, Richards (57), working in concentrated sucrose solutions (ca. 70 Brix) at 100°C and ca. 0.5% salt content observed increases in sucrose hydrolysis rates in the presence of salts. The addition of $MgCl_2$ effected a dramatic increase in hydrolysis rate, while $CaCl_2$ and NaCl addition also affected the rate to lesser extents, as shown in Figure 11. Richards proposed that the increase in hydrolysis rate was caused by withdrawal of electrons from the glycosidic oxygen by protonation with the hydrated magnesium ion, as shown in Figure 12. Sodium acetate, unlike sodium chloride, showed no increase in hydrolysis rate. Sodium acetate, where the cation is complexed with acetate rather than hydrated, is not capable of protonating the sucrose glycosidic oxygen and therefore would not, according to Richards' proposed mechanism, be expected to increase the rate of hydrolysis.

The increased rate of hydrolysis in ash concentrations in the 0.5% range reflects cane sugar refinery and raw sugar factory (sugar end) processing conditions more accurately than the rates in pure sucrose solutions of low concentration. Current research (12,57) shows that hydrolysis, or inversion, rates in refinery liquors and factory syrups are more rapid than earlier literature (49,50,60,61) indicated. Earlier studies did not simulate ash levels in processing conditions. Further research, on simulated refinery liquors and factory syrups, is indicated.

An important consequence of sucrose degradation is the development of colour from degradation products. Kurudis and Mauch (30) have developed an equation for the prediction of colour development in model sucrose solutions. Colour development was expressed as a function of temperature (90 to 120°C), time (0 to 80 min), pH (7.5 to 9.5), and composition of the solution (sucrose 20 to 60%, invert 0.02 to 0.18% and amino acids 1 to 3g/L). The authors claim with caution that the effects of an intended alteration in a unit process in the refinery can be predicted in advance.

Vukov (66) has developed equations based on experimental data that predict the effect of temperature, pH and ionic strength on rate constants of sucrose decomposition in acid and alkaline medium. Other workers (21) report that Vukov's equation generally agrees with their experimental rate data.

The literature on sucrose loss in manufacture is extensive, and not all studies have been conducted on the principles of sound scientific method. An example from the literature of a study with

questionable results is reported by Cecil (10), on the effect of pH
on sucrose loss during boiling in the open pan sulphitation (OPS)
process. "Juice" (massecuite from Kenyan OPS mill diluted to 20
Brix in a laboratory in the U.K.) was reclarified and re-evaporated
by a method "comparable to that used in an OPS mill." Sucrose:
potassium and invert:K ratios were used to determine sucrose loss;
in this way data were corrected for volume changes. Reducing
sugars and sucrose (by difference after acid hydrolysis) were
determined by the Lane-Eynon method and K, by AAS. Cecil claimed
that no measurable sucrose loss was detected between pH 6.5 and
7.5. Considering that oligo- and polysaccharides interfere with
the sugar analysis method and that some K must have been lost to
the clarifier mud, the results could actually have shown a net gain
of sucrose in the process. More direct quantitative methods (e.g.,
HPLC or HPIC) should have been used, although the application of
technology such as AAS or HPLC to open pan boiling is questionable
in itself.

At the other end of the technological spectrum is the use of
statistical process control (SPC) by British Sugar in beet sugar
factories to achieve better control of continuous plant operation
(58). Until recently SPC had mainly been used in batch processes
with unit entities as products; measurement of a sample of product
entities followed by statistical analysis indicated turning points
in the process. The application of this method to a continuous
product stream required a larger sample size to identify turning
points requiring corrective action. Parameters under control
included cossette quality, pH between 1st and 2nd carbonatation,
thick juice pH, waste condensate temperature, and standard liquor
colour (these factors affect sucrose hydrolysis and loss). This
type of experiential control does not identify the cause of
deviation from acceptable parameter values. The aim of SPC is to
identify turning points, initiate corrective action and re-
establish control of the process. British Sugar reports great
success with SPC in the last two sugar campaigns.

A recent symposium (62) on refinery losses - measurement and
control revealed some of the difficulties in correlating literature
reports of plant operations with one another, and even more so with
laboratory studies. A basic terminology problem exists with the
use of the terms "sucrose" and "pol" to mean the same in some
cases, and to mean different measurements in others. Considerable
variation was found in the basics of loss reports, only 1/3 of
which (from 29 companies responding to survey) actually called the
report a "loss statement." Loss was measured as a fraction of
"melt solids" by only half of the survey respondents; others used
"melt weight", "melt pol" or other denominators (67). Furthermore,
even the raw sugar weight factor was not consistent; half the

respondents used settlement scale, and half melt scale, with the
difference between the two ranging from 0.1% to 1.0% (figures that,
perhaps not coincidentally, represent net or unknown sucrose loss
in many refineries) (67).   In experimental studies, the term
"sucrose loss" generally refers to chemical sucrose loss on raw
sugar solids input. In manufacturing, a point usefully emphasized
by the symposium survey, chemical, physical, and accounting losses
are all combined (67). Two refineries could show identical "loss"
figures; one could be real losses on packaging over-weights and/or
chemical loss; the other could be a "paper" loss from incorrect
melt weights and product weights. The variety of materials weighed
at different plants makes consistent mass balance calculations
almost impossible, even more so in factories than in refineries,
though in many countries, factories within a given area will use
consistent reporting practice.

A useful summary of sucrose losses under good conditions was
presented in the Symposium by Latham (34), as follows, for percent
on melt solids:

    Loss in carbonatation filtration: < 0.01%
    Loss in phosphatation (scum loss): 0.01% - 0.02%
    Loss in bone char (wash + burn): 0.07%
    Loss on carbon (equivalent decolorization use to char): 0.08%
    Loss on resin (equivalent decolorization use to char): 0.03%
    Loss in condenser water: <0.01%
    Loss in packaging (overweights): 0.09%

The symposium was especially useful in pointing out the multitude
of factors that can lead to, or be included in, "sugar loss."

The control and measurement of pH.

In sugar refinery control operations, pH electrodes should not be
calibrated with standard buffer solutions and then placed on stream
in sugar liquors and assumed to read equivalent pH. Implicit in
this operation is the equivalence of pH electrode response in
dilute aqueous buffers at ca. 24°C and in high Brix sugar solutions
at elevated temperatures. Such equivalence does not exist.

Even in the case of laboratory research with dilute sucrose
solutions the measurement of pH at high temperatures is affected
not only by the change in water activity ($K_w = 10^{-14}$ at 24° and 5.13
x 10^{-13} at 100°C or $pK_{water} = 12.29$ at 100°C) (39), but also by a
change in the characteristics of the electrode used in the
measurement. In investigations where a pH electrode is calibrated

at 25° and pH measurements are made at much higher temperatures the differences between calibration and operation temperatures certainly results in some error in the pH value. Furthermore while many pH electrodes may give stable pH readings even in boiling alkaline solutions, the relationship between these observed pH values and the actual hydrogen ion activities are often undetermined.

The measurement of pH is further complicated by the effect of high concentrations of sucrose (e.g., 60 Brix or 60%wt/wt) on hydrogen ion activity. Clarke (11) has discussed the effect of sucrose solution structure on pH and calcium ion electrode processes and shown a decreased response of these electrodes to changes in ionic activity in sucrose solutions at 60 Brix and 24°C. This reduced electrode response can in part be explained by the structural order of the sucrose-water mixture (molecular association in sucrose-water systems has been reviewed by Allen et al.) (1). In a 60 Brix sucrose solution the ratio of water molecules to sucrose molecules is 12.7:1, with water molecules hydrogen-bonded to sucrose (i.e., in the solvation shell) in dynamic equilibrium with free water. Therefore, the concentration of free water molecules and dissociated ions is much less than in dilute sucrose solutions. The number of water molecules in the sucrose solvation shell is dependent on the extent of intramolecular hydrogen bonding in the sucrose molecule. The effect of sucrose concentration and temperature on intramolecular hydrogen bonding in aqueous sucrose solutions is still not completely resolved, although recent advances (51,54) in molecular modelling suggest that sucrose has far more freedom of rotation about the glycosidic linkage (i.e., less intramolecular hydrogen bonding) than previously thought (69).

Therefore in most cases pH values measured at high temperatures in dilute solution should be considered approximate values only. In cases where the investigators address this problem and are careful to select a suitable electrode (viz., one that manufacturers claim to have almost hysteresis-free pH measurement and a stable isopotential point over the temperature range) the error associated with electrode performance will be small, and differences in reported pH values will correspond to differences in actual pH. In cases where pH is measured in concentrated sucrose solutions the reported pH value should be considered as a nominal value only, and the differences in nominal pH values may or may not correspond to actual differences in hydrogen ion activity.

In process control, it is change in pH that is monitored to show gain or loss across a unit process, and to show trends. Regular recalibration of pH electrodes is essential for good control operation. Van der Poel et al. (65) in 1982 described progress in

development of computer and on-line control in CSM Suiker B.V. beet sugar factories. Control methods in all aspects of factory operation were described; for the purpose of this review control of pH during juice purification will be discussed. In addition to high temperature glass electrodes inserted in the process stream, CSM installed electrodes in a thermostated by-pass to measure pH at a lower temperature. CSM recognised that readings taken from these electrodes were nominal pH values only. Short term decisions to adjust alkali concentrations with soda ash or NaOH were based on the electrode response in a control loop with pH value set points. The set points were regularly adjusted by using laboratory acid-base titration results so that problems with calibration of the electrodes were avoided.

Based on the above inadequacy of pH measurements we stress a cautious approach in the interpretation of fundamental research data in the industrial environment. For example it would be unwise to conclude that a nominal pH value (from a pH electrode in a sugar refinery) greater than 8.5 indicates hydrogen ion activities that are too low to catalyze sucrose hydrolysis at a significant rate. The net gain or perhaps even the lack of a net loss of invert across a unit process is the best indicator of acid hydrolysis. The use of pH electrodes in the sugar industry should be based on experiential evidence that maintenance of nominal pH above a minimum value will prevent sucrose loss to invert.

SUMMARY

The mechanisms of sucrose, glucose and fructose degradation, in aqueous solution across a wide pH range, are outlined and applications to sugar refinery and factory situations are discussed.

Alkaline decomposition of sucrose can proceed by either (1) hydrolysis to glucose and fructose, by the normal acid hydrolysis mechanism (up to ca. pH 8.5), literature has perpetuated the false concept of "alkaline hydrolysis"; or (2) internal rearrangement to yield ionic forms of reducing disaccharides which proceed directly to acids, with no formation of invert. Recent intensive work on the alkaline degradation of monosaccharides is outlined. Mono-saccharides (glucose and fructose) first form an enediol anion which yields "degradation products" by any of five pathways: β-elimination, benzilic acid rearrangement, α-dicarbonyl cleavage, aldolization or retro-aldolization. These reactions and products are correlated with invert formation and loss and colour gain in the carbonatation, filtration and evaporation processes.

SPRI

The most probable mechanism for acid hydrolysis of sucrose is discussed; degradation pathways of monosaccharides at acid pH are outlined, including that to HMF. Other reaction products are listed. The importance of Maillard reactions at acid pH is emphasized, although reactions are not detailed here. The effects of difructose dianhydrides are explained.

The effects of these various sets of reactions are related to process steps in sucrose manufacture. Literature inaccuracies are traced, and current insight on the effect of ash and solids concentration on hydrolysis rates are described. The multiplicity of contributing factors to sugar loss is emphasized; types and magnitudes of refinery losses are classified.

The necessity of good pH control is emphasized, and common problems with electrode calibration and subsequent effects on control are discussed.

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Table 1. Rate of degradation of sucrose and model compounds.

Compound	10^4 x initial rate[a] (mol L^{-1} hr^{-1})
Sucrose	19
Methyl β-D-fructofuranoside [1]	< 0.1
Methyl α-D-glucopyranoside [4]	< 0.1
Octa-O-methylsucrose [7]	< 0.1
Methyl α-D-fructofuranoside [2]	20
Methyl 3,4-O-dimethyl-α-D-fructofuranoside [3]	< 0.1

a. 6.0 mM in 1 M NaOH at 100°C.

G.W. O'Donnell, G.N. Richards (1973)

Table 2. Reported products of fructose decomposition in water at elevated temperatures.

Dehydration	Fragmentation	Condensation
5-(hydroxymethyl)-2-furaldehyde[a]	formic acid[a]	"humin"
5-methyl-2-furaldehyde[b]	levulinic acid[a]	
α-angelica lactone[b]	dihydroxyacetone[a]	
β-angelica lactone[b]	glyceraldehyde[a]	
2-(2-hydroxyacetyl)furan[b]	2-furaldehyde[a]	
2-(2-hydroxyacetyl)furan formate[b]	pyruvaldehyde[a]	*Isomerization*
isomaltol[b]	lactic acid[a]	
4-hydroxy-2,3,5-hexanetrione[b]	acetol[a]	
4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2H)-furanone[b]	glycoaldehyde[b]	D-glucose[a]
	acetic acid[b]	
	2,3-butandione[b]	

a Major products (generally > 1% absolute yield). b Minor products

M.J. Antal, Jr., W.S.L. Mok, G.N. Richards (1990)

Figure 1.

*The mechanism of alkaline degradation of β-D-glucopyranosides.*
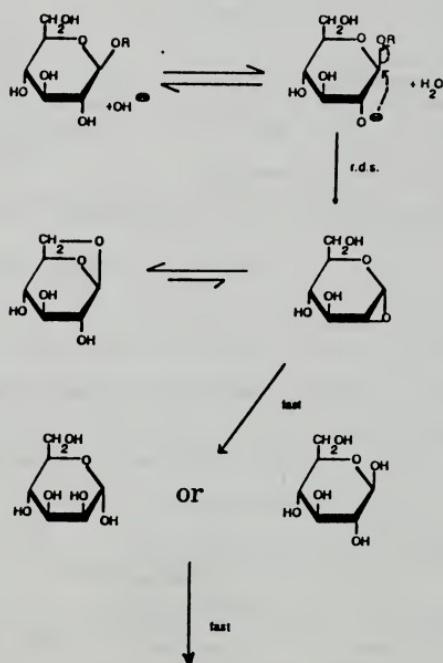
Degradation Products

Figure 2.

The alkaline degradation of sucrose via *the $S_N iCB$ mechanism of Richards* et al.
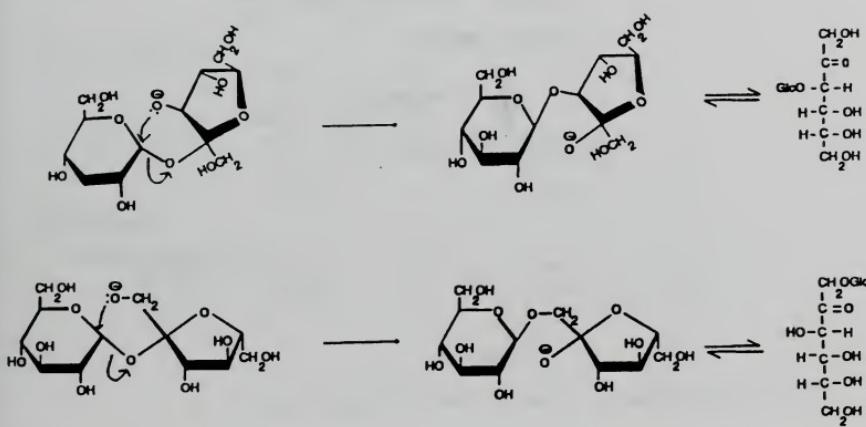
M. Manley-Harris, G.N. Richards (1981)

Figure 3.

Simplified overall reaction scheme of monosaccharides in alkaline medium.

MONOSACCHARIDE

↑↓ ionization, mutarotation

MONOSACCHARIDE ANION

↑↓ enolization, isomerization

ENEDIOL ANION

↓ "degradation"

CARBOXYLIC ACIDS
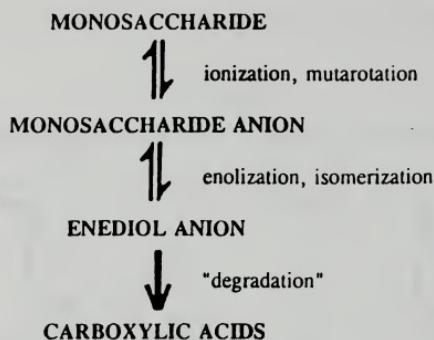
J.M. de Bruijn (1986)

*Figure 4.*

Mutarotation and isomerization of reducing sugars.
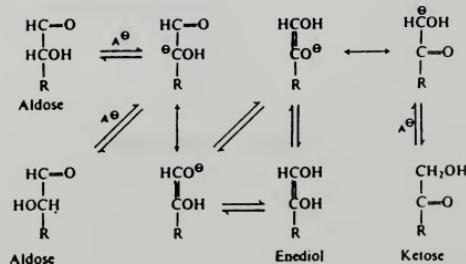
*Figure 5.*

(I) β-elimination
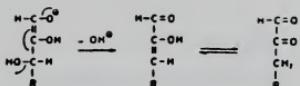
(II) benzylic acid rearrangement
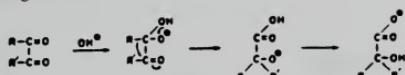
(III) α-dicarbonyl clevage
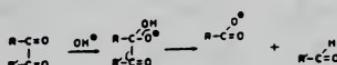
(IV) retro-aldolization
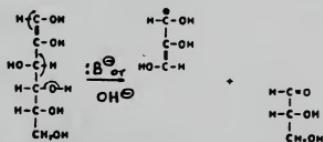
(V) aldolization
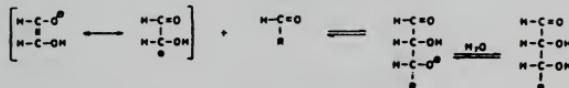
J.M. de Bruijn (1986)

Figure 6.

*Reaction model for the alkaline degradation of reducing sugars.*

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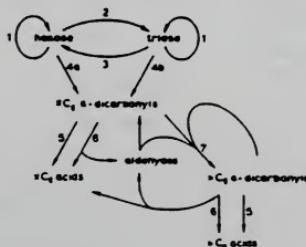
Figure 7.

*The mechanism of acid hydrolysis of sucrose.*
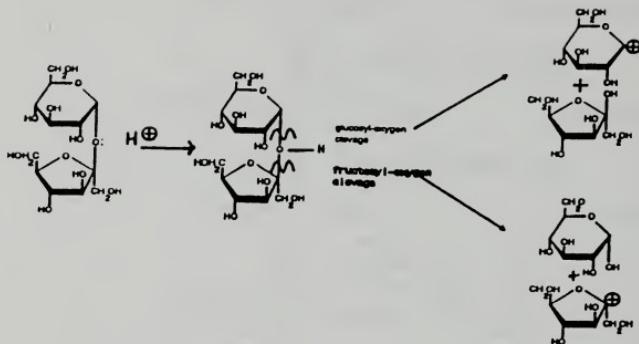
Figure 8.

*Enolization of reducing sugars in acid solution.*

348
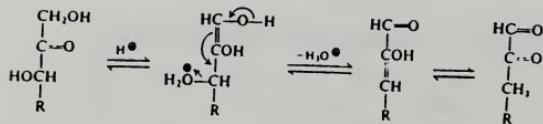
Figure 9.

Carbonyl-amine reactions leading to Maillard reaction products and reductones.
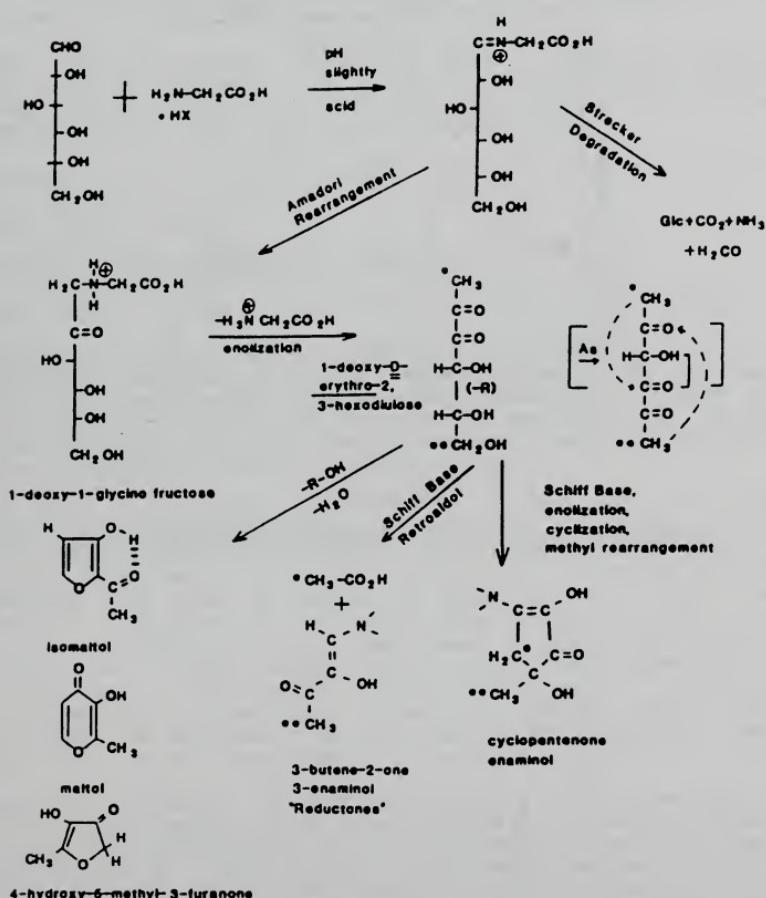
Figure 10.

The effect of chloride salts on sucrose hydrolysis rate.
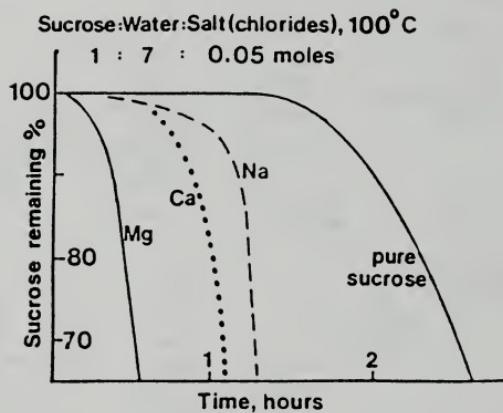
Figure 11.

Catalysis of glycosidic oxygen protonation by hydrated magnesium ions.

350
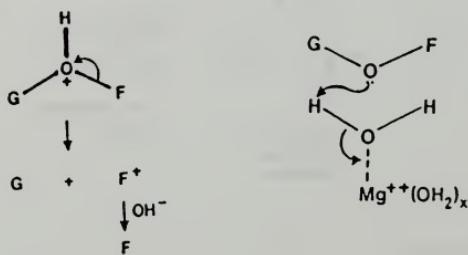
DISCUSSION

Question: Congratulations on this good work. You showed a slide on the influence of salts on the rate of acid hydrolysis (Figure 10). It looks like there is a lag phase in the degradation - could you comment on that, please?

Edye: The pure sucrose solution is about 70 Brix (one sucrose to seven water molecules) and is a neutral solution. The products of decomposition catalyze the further degradation of sucrose by lowering the pH. The reaction is autocatalytic.

Question: So the reactions are carried out without pH control?

Edye: That's right.

Question: What was the pH of that test?

Edye: It was the pH of 70 Brix sucrose/water: it's about 8.

Question: I would like to endorse the comment in the first question of the value of this work. For all of us who work practically in the sugar industry, the measurement of sugar loss is absolutely crucial to the finances of our business. A current review of actual conditions and progress has been long outstanding, since Ken Parker's review in the mid-70's.

I do have a question: for those of us who rely on a method, introduced by British Sugar many years ago, to measure invert by triphenyl tetrazolium chloride, the deductions made about losses are quite incorrect. In alkaline media, you are losing sucrose and measuring the loss as though it were invert by the chemical methods. Nowadays, many people are using enzymatic glucose analyzers and then doubling the answer to call that invert. My question is: in this review, are you going to discuss differences in the rates of glucose and fructose reactions? We talk about invert but of course that is made up of two quite different molecules. Is the answer we get from enzymatic glucose analyzers valid if we double that answer and call it invert?

Edye: In my opinion, at this time, there is really no significant alkaline degradation _per se_ of sucrose in factories and refineries. The mechanism I showed is at very high pH's and temperatures. The issue at pH 8 or 9 (as measured by pH electrodes, and not necessarily the correct pH) is that there is acid hydrolysis occurring to form glucose and fructose. This enables subsequent alkaline degradation of glucose and fructose, because of course they are much more reactive at those pH's. The relative reactivities of

glucose and fructose at alkaline pH are discussed in the papers of J.M. deBruijn, A.P.G. Kiebnoom, H. van Bekkum, P.W. van der Poel, Sugar Techn. Rev., 13 (1986) 21-52.

de Bruijn: I agree, at pH 8 to 9 there is still acid hydrolysis of sucrose. In our experience, at those pH's and at the temperatures of evaporation, not much alkaline degradation is observed.   At least, we did not see an increase in lactic acid.

In the vacuum pan station, at higher Brix, reactions of invert with amino acids occur - the Maillard reactions.   These are not discussed in my own paper.

Edye: With regard to measurement of sucrose loss by the enzymatic glucose detectors, I believe that these detectors are unable to measure glucose (and fructose) in very high sucrose concentrations, in which glucose oxidase is inhibited.   Ion chromatography procedures which measure sucrose, glucose and fructose individually and are very sensitive, are probably more suitable for measuring sucrose losses in process.

POLYSACCHARIDES OF BEET AND CANE SUGAR: A PROGRESS REPORT

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ABSTRACT

This presentation summarizes work on polysaccharide nature,
structure and occurrence.

Effects of various polysaccharides in sugarbeet processes,
including molasses desugarization,and effects on product quality
are outlined.

Origins of sugarcane polysaccharides, and their influence on
process efficiency and product quality are summarized.

INTRODUCTION

This paper summarizes work done by the SPRI group, and visiting
scientist J.M. de Bruijn, in the two years between the 1990 and
1992 meetings. Some of the work reported herein has been reported
in part in papers written during the relevant time period, and is
duly referenced. The emphasis is on polysaccharides of sugarbeet,
a field which has been heretofore investigated to a lesser extent
than that of sugarcane polysaccharides. Nevertheless, even after
the many projects and publications on sugarcane polysaccharides,
both plant and microbial in origin, there are still unanswered
questions about their effects in process and on analytical
measurements.

SUGARCANE POLYSACCHARIDES

The variety of naturally occurring sugarcane plant polysaccharides
(e.g. dextrans) were most recently reviewed in 1986 (2) and 1988
(11). Since that review, phoytoglycogen has been identified as a
plant polysaccharide of sugarcane (18).

Polysaccharides have been proposed as part of the very high
molecular weight (VHMW) ($2x10^6$ daltons) color complex, that is

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difficult to remove in sugar refining, and that transfers preferen-
tially to the crystal (8). Data in Table 1 indicates the relative
amounts of high molecular weight color (>12,000 daltons) and total
color transferred to crystal, in studies on several raw sugar
factories, and point out that HMW color is transferred to crystal
in a higher percentage than total color. Table 2 shows transfer of
starch and total polysaccharide to crystal; both polysaccharides
transfer at a much higher rate than colorant or ash components.

Recent investigations using ^{13}C nmr (nuclear magnetic resonance)
spectroscopy have confirmed the presence of polysaccharide in VHMW
colorant; the polysaccharide may be responsible for the increased
rate of transfer to crystal. Table 3 shows the chemical shifts
observed in the ^{13}C nmr spectra compared to dextran standard (from
Leuconostoc mesenteroides B-512F). The spectra of dextran and VHMW
colorant shown in Figure 1 indicate the presence of dextran, but
also of some other polysaccharide. The signal at 61.51, which
represents the anomeric carbon in non-reducing glucose units, is
generally interpreted as an indicator of branching points (4).
However, its intensity is here as strong as that of the anomeric
carbon on reducing glucose units, indicating either a very high
degree of branching (since this material is all of molecular weight
>12,000 DA) or some unidentified other signal at that frequency.
This sample was difficult to dissolve for nmr, and so the apparent
dextran may not be representative of the whole complex but only of
the soluble portion. The proton nmr's confirm the presence of
dextran.

The difficulties in removing polysaccharides from process by any
treatment other than enzymic hydrolysis, or purification by
crystallization, are well recognized (9,12). An example is shown
in data in Table 4, from a factory making raw and plantation white
sugar (by juice sulfitation) and "special" white sugar, made by
recrystallizing the standard plantation white. It can be seen that
the standard plantation white contains similar levels of starch and
polysaccharides to the raw: only the color and phenolics have been
reduced by sulfite bleaching. The "special" white, which has been
recrystallized, shows a decrease in levels of starch and polysac-
charides.

SUGARBEET POLYSACCHARIDES

The types and relative occurrence of sugarbeet polysaccharides are
outlined in Table 5. A review of composition and structure of
sugarbeet polysaccharides has recently been compiled by the senior
author (5,6), and is excerpted here:

The polysaccharides of sugarbeet were for many years, in common with most plant polysaccharides, an art or a craft, rather than a subject for systematic scientific investigation. References are found in older literature to "pectic substances," generally said to be composed of pectic acids, hemicellulose, and various homo-polysaccharides, such as araban and galactan. Pectin was recognized as a polysaccharide (a homopolygalacturonan), but since sugarbeet pectin is highly acetylated and has low gelling power, was regarded only as a viscosity problem in processing and a cause of false pol (13,14,21).

In recent years, with the growing importance of by-products or alternate products from sugarbeet, and the emphasis on the effect of non-sugars on yield and factory efficiency, polysaccharides have received greater attention.

McCready in 1966 summarized the chemistry of sugarbeet pulp polysaccharides. Since that review, the work of Vogel and Schiweck in Germany (23,24), of Chang's group in North Dakota (15,25) and of our own group, SPRI (3,10), has contributed basic structural and chemical information on the nature of the polysaccharides in sugarbeet. Development of beet pulp as a dietary fiber has lent impetus to this work, and is the other reason why most analyses are reported on pulp rather than on beet.

The seminal work of J.F. Thibault (19,22) and co-workers on sugarbeet pectins has given a better understanding to the structure of that fraction than to that of any other sugarbeet polysaccharides.

STRUCTURE OF PECTINS AND HEMICELLULOSE

Pectin

Pectins from sugarbeet pulp range in molecular weight from 15,000 - 48,000 daltons, with a backbone of α-(1,4) linked α-D-galacturonic acid residues containing a small proportion of α-(1,2) linked of L-rhamnose residues. Side chains of neutral sugar occur in the so-called "hairy" regions of the backbone, leaving unsubstituted "smooth" areas. The side chains, usually attached to C-4 of the rhamnose residues, are of α-(1,5)-L-arabinofuranosyl residues and β-galactosyl residues (probably 1,4 linked). The large number of acetyl groups (up to 35% acetylation) that are responsible for the low gelling power of sugarbeet pectin, are mostly linked to C-2 or C-3 of galacturonic acid units in the "smooth" regions. Ferulic acid ester groups are attached to the "hairy" pectins, on the side

chains, some esterified to arabinose units, the rest presumably to galactose units (22).

Hemicellulose

By definition, hemicelluloses, which are non-cellulose carbohydrate polymers, are considered to include pectins, but because pectins are such an important part of the sugarbeet hemicellulose, they are considered separately. Older literature (13,14,21) refers to hemicellulose as made up of "arabans" and "galactans." The authors have not found any reports of isolation of pure compounds corre- sponding to polymers of arabinose or of galactose and suggest that these names were applied to extracted hemicellulose material that was shown to contain arabinose and galactose units. Extraction procedures are often difficult to reproduce; results on composition and hydrolysis products can vary widely, even when similar extraction and hydrolyses procedures are used. Pectins dissolved from the alcohol-insoluble residues of sugarbeet pulp have been well characterized (22); it is presumed that the remainder of the alcohol-insoluble residue is cellulose and other hemicelluloses.

It appears quite probable that the hemicellulose material is galactoarabinan in nature, with varying degrees of composition. The difficulties in separating pectins from hemicellulose compound the problems of analysis: the "hairy" regions of pectins have been shown to contain some α-(1,5) linked arabinose chains (22). Incomplete separation of various fractions is no doubt responsible for the variety of reports of composition and structure of sugarbeet hemicellulose. Araban (more correctly called "arabinan") is said (13,25) to be highly branched, with an α-(1,5) linked backbone and α-(1,3) linked side chains. If this is so, then, because the arabinose is in the furanose form, the backbone might have a helical structure, and offer the possibility of acting as an RNA analogue for nucleoside synthesis. Many arabinofuranases are available: if the backbone is indeed made up of only arabinose residues, and if the correct arabinofuranases can be identified, then the compound offers not only synthetic potential, but a higher-volume use as an unbranched linear polysaccharide for food technology application. The latter potential is limited by the molecular weight, reported variously as 8,700 - 24,200 daltons and 18,400 to 37,000 daltons (25).

The total sugars composition of the hemicellulose fraction has been reported from several sources (3,23) with general agreement, as shown in Table 3. It is logical to expect variations in beets from different sources; the agreement between hemicellulose from thin juice (23) and raw juice with pectins removed (3) is surprisingly

good. Studies by Vogel and Schiweck (23) have shown that pectins are degraded or removed almost completely during juice purification, and so the hemicellulose in thin juice is essentially pectin free.

Molecular weight of this fraction has been reported as between 100,000 and 300,000 (23), where material greater than 10,000 daltons is removed during the isolation process. This agrees with the molecular weight determination of pulp hemicellulose B fraction of 150,000 daltons (25). Work at SPRI on high molecular weight colorant shows a fraction at 300,000 daltons that is found in white sugar and process materials (3,10); this colorant has polysaccharide nature and is thought to be related to hemicellulose material.

It is proposed that this hemicellulose polysaccharide is a galactoarabinan in nature, with an arabinose backbone. Separation, isolation and purification treatments by various workers have broken these cell-wall polysaccharides, which are very soluble, into different fractions, sometimes reported as "araban" and "galactan." The galactoarabinan structure could account for the diversity in reports of polysaccharides in sugarbeet.

The observations on hemicellulose molecular weights do not account for the very high molecular weight material with colorant nature that has been observed in white sugar and molasses (3,10). The high molecular weights of 800,000 - 1,000,000 daltons, are indicative of microbial polysaccharides, but these would not be expected to have colorant nature. However, the presence of dextran in the very high molecular weight material from sugarcane, described above, may have an analogous situation in beet colorant.

Hemicellulose composition

Polysaccharides have now been isolated from sugarbeet juice from several areas. Composition of these polysaccharides shows greater variety than originally thought, (3,23), and is reported for several samples in Table 8. These appear to be the hemicellulose fraction combined with microbial polysaccharides.

Polysaccharides in B-syrup (from a low purity second strike) were isolated by several methods (dialysis, alcohol precipitation, and ultrafiltration). Other high molecular weight components of tenate (material remaining in dialysis tube) are: proteins, colorant, and inorganic material that may be covalently bonded to the polysaccharides. Analysis of the trifluoroacetic acid hydrolyzates of these tenates are reported in Table 8.

The B-syrup polysaccharides isolated by ethanol precipitation were also analyzed, by methanolysis (17) for uronic acids. No galacturonic acid residues were observed, indicating that no pectin has survived through processing to this step. Pectins removal is one goal of juice purification: Vogel and Schiweck (23) had reported small amounts of galacturonic acid residues in thick juice and molasses. The efficiency of removal will vary among factories, and may also vary with the molecular weight of uronic acid-containing fragments.

No glucuronic acid was observed in the polysaccharide. Glucuronic acid residues have not been reported in sugarbeet polysaccharide. However, glucuronic acid residues on sugarcane polysaccharide are known to be responsible for cane acid beverage sugar floc (3) so their presence was of interest.

General changes noted across processing include:

1) Decrease in glucose levels, possibly because of removal of dextrans and other glucans in juice purification and filtration.

2) Removal of galacturonic acid residues, again most probably removed in juice clarification.

3) Arabinose: galactose ratio could be an indication of change in polysaccharide composition, but not enough data is available yet.

4) Loss of xylose, from raw juice to processed syrup, shows complete removal of sugarbeet fiber.

DISTRIBUTION OF TOTAL POLYSACCHARIDES IN BEET (5)

Significance of polysaccharide distribution

Knowledge of the location of polysaccharides in the beet root can be important for the following reasons:

1. The development of alkaline peeling methods requires knowledge of components that can be removed with the peel and need not be taken into account for subsequent purification steps.

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2. Polysaccharide content, nature and distribution are important for beet storage quality and freeze=thaw behavior.

3. Utilization of polysaccharides as byproducts can be made more efficient if compounds are found to be localized in one area of the beet root.

Paired samples of sugarbeet peel and sugarbeet interior were collected from beets stored at various times, temperatures, and conditions and were extracted by a cold water technique, to remove soluble polysaccharides and minimize pectin extraction. Duplicates of two of four replicates of each beet variety were used. The authors are grateful to the USDA-ARS Crops Research Laboratory, Fort Collins, Colorado for the samples (See ACKNOWLEDGEMENTS). Water extracts were depectinated by a lime slurry treatment, and analyzed for total polysaccharide, by the SPRI method (16).

Results on total polysaccharide are shown in Table 6. Each sample was analyzed in duplicate, so that each replicate had 4 analyses. Averages of sample duplicates are reported. Results in Table 6 indicate that pile stored beets, at lower relative humidity and very cold temperatures, develop much more polysaccharide in peel than beets stored under refrigeration at 4°C and high humidity.

The concentration of polysaccharide in peel is, as expected, an order of magnitude higher than that in the interior of the beet. Sugar content of the peel and interior samples are shown in Table 7. The possibility of false pol, particularly in the peel samples, from an extraction procedure designed for polysaccharide, not sucrose, must be kept in mind.

Other aspects of polysaccharide in processing discussed in the abovementioned review (5) include the association of polysaccharide with high molecular weight colorant; increase in polysaccharide content in sugar made by molasses desugarization, and the association of high levels of polysaccharides in white sugar with characteristic odors (often enhanced in storage) of white sugar. This last observation is further explained in a discussion of effects of beet polysaccharides in processing (6): microbial infection, which produces both strongly smelling short chain fatty acids and microbial polysaccharides, can account for the association. The short chain fatty acids (butyric, valeric, etc.) are relatively volatile, and can be concentrated in evaporator condensate. Condensate is often used in wash water on sugar, thereby transferring a relatively concentrated solution of high odor compounds to the final product.

MICROBIAL POLYSACCHARIDES

Microbial polysaccharides (levans and dextrans, polymers of, respectively, fructose and glucose) have often been reported in sugarbeet deteriorated by freeze damage or physical damage (14,21).

It has been thought that levans were the predominant species. Tests have been published for levans and dextrans and other polysaccharides in beet, although these are general non-specific thin-layer chromatography tests for polysaccharide hydrolyzates. The test for "dextran" in the "ICUMSA Methods" will give a positive result for any polysaccharide hydrolyzed by the conditions of the test (20).

Samples of sugars and process materials from freeze damaged and heat damaged sugarbeet were tested for dextran, using the AOAC method for dextran in raw cane sugar (preparation, in alkaline medium, of a copper complex of dextran; isolation of the copper-polysaccharide complex and determination of the polysaccharide content) (1). Levels of dextran observed in frozen and normal (not frozen) samples are shown in Table 10. Levels of dextran, and % of total polysaccharides, from heat damaged beet, are shown in Table 11.

It was necessary to check the AOAC test to see if, in sugarbeet samples, it tested for dextran only or for dextran mixed with other polysaccharides or glycoproteins. A high dextran white sugar, made from freeze-damaged beet, was the subject. The scheme used to isolate the copper-polysaccharide precipitate, remove the copper, and identify the precipitate remaining is outlined in Figure 3.

The white precipitate isolated by this procedure was hydrolyzed and analyzed for component sugars: it was >98% glucose, and therefore a glucan. The glucan was subjected to ^{13}C and proton nmr spectroscopic analysis, and shown to be similar in structure to standard dextran (Pharmacia Corp.) made from Leuconostoc mesenteroides B-512F (7). This is the same dextran found in deteriorated sugarcane and its products. It shows some 5% branching, and a high molecular weight (over $2x10^6$ DA) and makes an extremely viscous solution.

Dextrans were then isolated from several other sugarbeet products by the same procedure, and sent for nmr analysis. Sources of other dextrans were:

1.   A second sugar from freeze damaged beet (U.S. origin).

2.   A raw beet sugar (European origin)

3.   Juices from heat damaged beet (U.S. origin).

Comparisons of the nmr spectra assignments of these dextrans with standard dextran from L. mesenteroides B-512F are shown in Table 12.   The ^{13}C spectra are shown in Figure 4.   The C6' carbon is the anomeric carbon on the non-reducing glucose unit.   Its intensity (not shown here) indicate the concentration of branch points, or degree of branching of the polysaccharide.   Dextrans from samples 1,2 and 3 above have very little indication of branching.   Proton nmr spectra bear this out.   This structural investigation is continuing: if these are essentially unbranched dextrans, they could be a valuable product.

SUMMARY AND CONCLUSIONS

This paper has described recent findings on sugarcane polysaccharides, and shown their high rate of transfer from solution to crystal during sugar boiling.

The polysaccharides of sugarbeet are reviewed in summary, and work on isolation and identification of the hemicellulose fraction is described.   Occurrence of hemicellulose (IBP) throughout processing, and its effects on processing and products are outlined.

Microbial polysaccharides of sugarbeet, heretofore thought to have been principally levan, have been found to contain significant quantities of dextrans.   These dextrans have been isolated and analyzed by nmr spectroscopy.   They appear to be similar to the standard dextran produced by Leuconostoc mesentoides B-512F, and of high molecular weight.

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Table 1. Colour transferred into crystal. All colors in ICU
on solids.

		Syrup	A Sugar	% Transfer
Total	Fact. A	10,488	814	7.8
Color	Fact. B	20,773	1352	6.5
HMW	Fact. A	2347	290	12.4
Color	Fact. B	4720	529	8.3
Total Color	Fact. A	8141	524	6.4
-HMW Color	Fact. B	16,053	823	5.12
(Color <12,000 DA)				

Table 2. Starch and total polysaccharide transferred in crystal
(ppm on solids).

		Syrup	A Sugar	% Transfer
Starch	Factory A	604	203	33.6
	Factory B	740	279	33.7
Total	Factory A	4073	717	17.6
Polysac.	Factory B	2031	350	17.2

Table 3. Chemical shifts for dextran B-512F and VHMW colorant.

	Dextran - reference T-10	VHMW Colorant
C1	98.58	98.67
C2	72.27	72.24-72.48 (triplet)
C3	74.27	74.35
C4	70.59	70.67
C5	71.06	71.16
C6	66.73	66.72
C6[1]	61.52	61.52

Table 4. Raw, plantation white and recrystallized "special"
          from same factory.

Component	Raw	White (Standard)	White (Special)
Colour (pH7)	1109	181	128
Phenolics, ppm	271	31	29
Total polysacc, ppm	859	601	668
Starch, ppm	196	87	196

Table 5. Types of polysaccharides in sugarbeet.

	Type	% in beet pulp
A.	Cellulose	20-25
B.	Soluble: pectin	25-30
	Hemicellulose	30-40
	Starch, gums	<3
C.	Microbial: Levans, dextrans	

Table 6. Total polysaccharide levels in stored sugarbeet,
          ppm on solids.

Set 1. Freeze storage

Sample	D-1	D-2
Peel	18,762	22,728
Interior	2,262	1,250

Set 2. Pile storage

Sample	A-4	D-4
Peel	34,195	31,363
Interior	1,561	1,519

Table 7. Pol (sucrose) of stored beets, peel and interior.

Sample	Average pol (sucrose)
Freeze stored beets,	
peel	2.84
interior	12.34
Pile stored beets,	
peel	3.88
interior	14.49

Table 8. Composition of polysaccharide isolated from raw juice, thick juice, and B-molasses (% component)

COMPONENT	RAW JUICE	THICK JUICE	B-SYRUP
Arabinose	33.3	31	29
Galactose	35.3	22	21
Rhamnose	–	–	trace
Mannose	–	–	15
Xylose	2.1	–	–
Glucose	17.6	52	31
Uronic acid	Not analyzed	–	–
Unknown	11.5	–	–
Ara/gal ratio	0.93	1.4	1.4

Table 9. Soluble non-pectin non-glucan, polysaccharide (IBP)

Process material	Concentration
Raw Juice	0.3% – 0.5%
Thin Juice	0.07% – 0.05%
Thick Juice	0.04% – 0.2%
B-Syrup	0.2% – 0.4%

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Table 10.   Dextrans in sugarbeet factory samples

SAMPLE	DEXTRAN. ppm	
	From frozen beet	From normal beet
Cossettes	3354	289
Diffusion juice	1199, on solids	527, on solids
White sugar	1890	<100

Table 11.   Polysaccharides and dextran in raw juice (depectinized)
            from deteriorated beet (hot weather conditions)

Sample No.	Total polysaccharide, % on juice	Dextran % of total polys
1	0.20	56.2
2	0.12	26.5
3	0.13	59.8
4	0.13	37.5
5	0.20	89.1
Average	0.16	53.82

Table 12.   Comparison of nmr data on chemical shifts of sugarbeet
            dextrans with standard dextran

		Dextran			
	Reference dextran	White sugar, frozen beet	White sugar (1)	Raw beet sugar (2)	Juice from heat damaged beet (3)
C1	98.58	98.67	98.63	98.64	98.62
C2	72.27	72.37	72.30	72.33	72.29
C3	74.27	74.35	74.29	74.32	74.29
C4	70.59	70.67	70.63	70.65	70.62
C5	71.06	71.6	71.11	71.12	71.09
C6	66.73	66.805	66.77	66.78	66.75
C6^1	61.52	61.6(est)	59(est)	-	61(est)
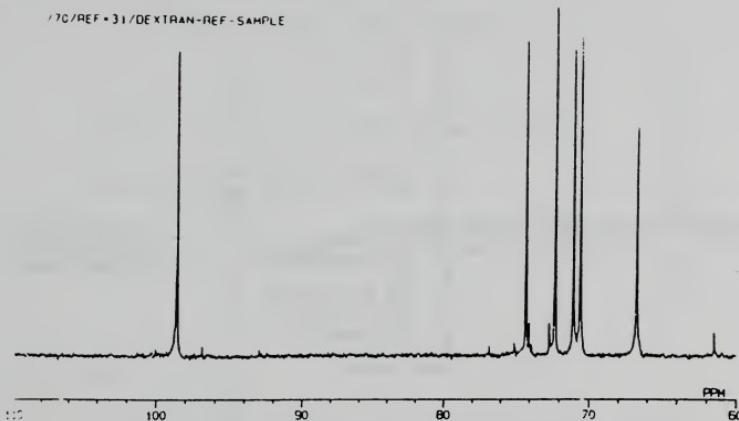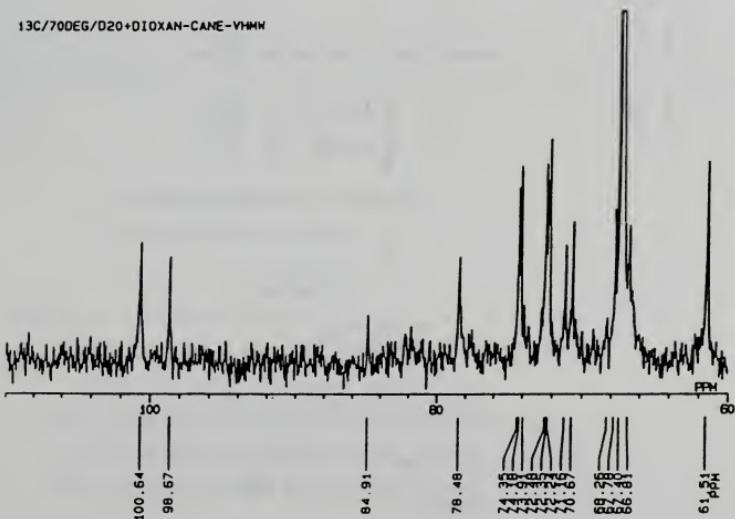
Figure 1. ^{13}C nmr spectra of VHMW colorant and standard dextran.

EXTRACTION SYSTEMS FOR SOLUBLE HEMICELLULOSE
(CELL WALL POLYSACCHARIDE)

SAMPLE (JUICE, SUGAR OR SYRUP)

Ca⁺⁺. pH 10
filtration, D.E.

DEPECTINIZED SAMPLE

Ethanol precipitation
DEAE-cellulose decolorization

Dialysis
(12,000 MW)

PRECIPITATED
POLYSACCHARIDES
GPC
TFA HYDROLYSIS
METHANOLYSIS
HPLC AND GC

Ultrafiltration
(30,000 MW)
Freeze-drying

COMPONENTS
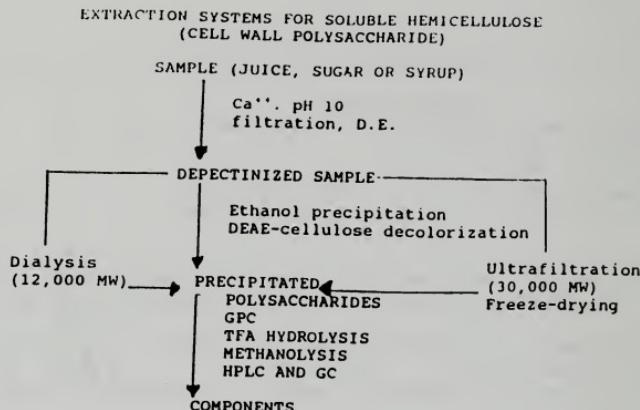
Figure 2.   Extraction systems for soluble polysaccharide.

BEET SUGAR SOLUTION OR JUICE

ALKALINE

COPPER (II)

COPPER-POLYSACCHARIDE COMPLEX

- DISSOLVED IN ACETIC ACID

- DIALYZED
- FREEZE DRIED

WHITE PPTE
HYDROLYZED
>98% GLUCOSE BY GLC

PRECIPITATE IS ALL GLUCAN.
LEVANS ARE NOT ISOLATED BY THE AOAC TESTS.

IS THIS GLUCAN SIMILAR TO CANE DEXTRANS?

GLUCAN SUBJECTED TO NMR METHYLATION GC-MS
ANALYSIS
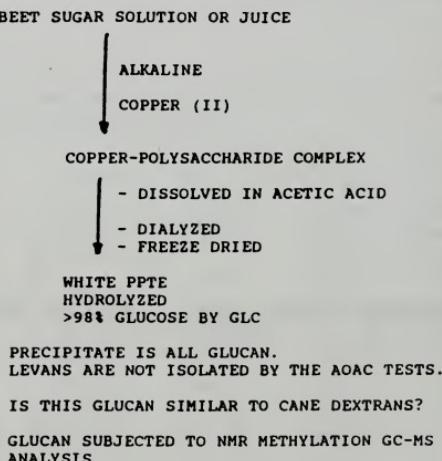
Figure 3.   Isolation of dextran from sugarbeet products.
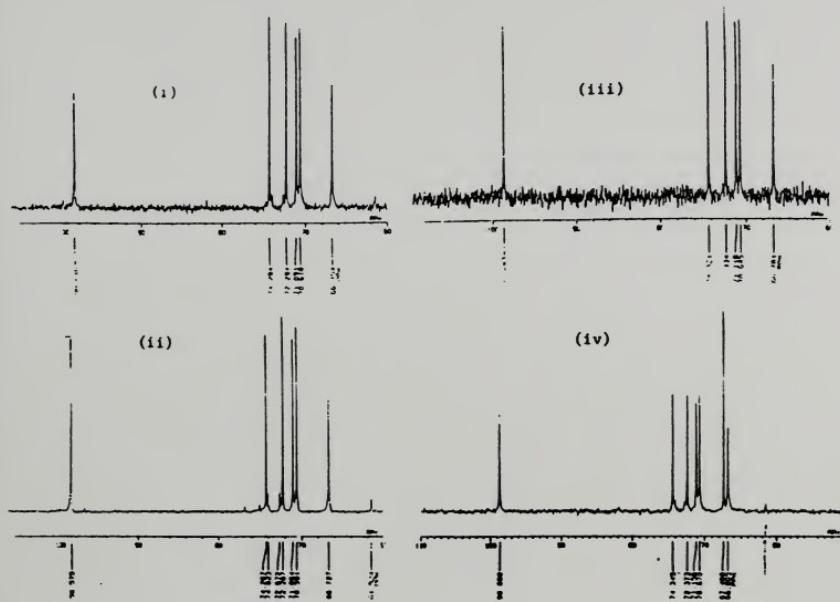
Figure 4. ^{13}C nmr spectra of

  (i)   dextran isolated from sugarbeet juice

  (ii)   standard reference dextran T-10

  (iii) dextran isolated from raw beet sugar

  (iv)   dextran isolated from white sugar made
         from frozen beet